EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	855	GDF-8 or BMP-11	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:31
L2	6	myo29	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:37
L3	5	myo-029	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:41
L4	50	GDF and (veldman.in. or davies.in. or song.in. or wolfman.in. or bridges.in. or royston.in. or russell.in. or valge?. in.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:43
L5	222	myostatin	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:43
L6	988	1 or 5	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:43
L7	159	6 same (antibod$)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2006/12/07 15:43

Welcome to DIALOG

Dialog level 05.14.00D
? b 411;set files biotech
07dec06 17:09:39 User219511 Session D666.2
$0.00 0.100 DialUnits File410
$0.00 Estimated cost File410
$0.02 TELNET
$0.02 Estimated cost this search
$0.43 Estimated total session cost 0.218 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
(c) 2006 Dialog

*** DIALINDEX search results display in an abbreviated ***
*** format unless you enter the SET DETAIL ON command. ***
You have 26 files in your file list.
(To see banners, use SHOW FILES command)
? s (gdf-8 or BMP-11 or myostatin) and antibod?

Your SELECT statement is:
s (gdf-8 or BMP-11 or myostatin) and antibod?

Items	File
34	5: Biosis Previews(R)_1969-2006/Dec W1
6	24: CSA Life Sciences Abstracts_1966-2006/Oct
28	34: SciSearch(R) Cited Ref Sci_1990-2006/Dec W1
9	45: EMCare_2006/Dec W1
17	71: ELSEVIER BIOBASE_1994-2006/Dec W1
44	73: EMBASE_1974-2006/Dec 07
2	94: JICST-EPlus_1985-2006/Aug W3
3	98: General Sci Abs_1984-2006/Nov
8	135: NewsRx Weekly Reports_1995-2006/Dec W1
1	136: BioEngineering Abstracts_1966-2006/Oct
1	143: Biol. & Agric. Index_1983-2006/Oct
6	144: Pascal_1973-2006/Nov W2
28	155: MEDLINE(R)_1950-2006/Dec 05
1	172: EMBASE Alert_2006/Dec 07
19	266: FEDRIP_2006/Aug
29	357: Derwent Biotech Res.__1982-2006/Dec W2
2	369: New Scientist_1994-2006/Sep W3
24	399: CA SEARCH(R)_1967-2006/UD=14524

18 files have one or more items; file list includes 26 files.

? save temp; b 155,5,73,357;exs;rd
Temp SearchSave "TC343178839" stored
07dec06 17:10:29 User219511 Session D666.3
$1.69 0.638 DialUnits File411
$1.69 Estimated cost File411
$0.26 TELNET
$1.95 Estimated cost this search
$2.38 Estimated total session cost 0.856 DialUnits

SYSTEM:OS - DIALOG OneSearch
File 155:MEDLINE(R) 1950-2006/Dec 05
(c) format only 2006 Dialog
*File 155: The file has resumed updating with UD20061120,
with RT=IN DATA REVIEW and RT=IN PROCESS records.
File 5:Biosis Previews(R) 1969-2006/Dec W1
(c) 2006 The Thomson Corporation
File 73:EMBASE 1974-2006/Dec 07
(c) 2006 Elsevier B.V.
File 357:Derwent Biotech Res. _1982-2006/Dec W2
(c) 2006 The Thomson Corp.

Set	Items	Description

Executing TC343178839
HILIGHT set on as '%'
15 GDF-8
5 BMP-11
1313 MYOSTATIN
1978874 ANTIBOD?
S1 135 (GDF-8 OR BMP-11 OR MYOSTATIN) AND ANTIBOD?
S2 95 RD (unique items)
? t s2/7/1-95

2/7/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

22084921 PMID: 17100637
Inhibitors of the TGF-beta superfamily and their clinical applications.
Tsuchida K; Sunada Y; Noji S; Murakami T; Uezumi A; Nakatani M
Division for Therapies against Intractable Diseases, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-1192, Japan. tsuchida@fujita-hu.ac.jp
Mini reviews in medicinal chemistry (Netherlands) Nov 2006, 6 (11) p1255-61, ISSN 1389-5575--Print Journal Code: 101094212
Publishing Model Print
Document type: Journal Article; Research Support, Non-U.S. Gov't
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
The transforming growth factor-beta (TGF-beta) superfamily includes TGF-betas, activin, %myostatin% and bone morphogenetic proteins. Misregulation of the activity of TGF-beta family members is involved in pathogenesis of cancer, muscular dystrophy, obesity and bone and tooth remodeling. Natural inhibitors for the TGF-beta superfamily regulate fine-tuning of activity of TGF-beta family in vivo. In addition to natural inhibitors for the TGF-beta family, soluble forms of receptors for the TGF-beta family, blocking monoclonal %antibodies% and small chemical TGF-beta inhibitors have been developed. In this review, we summarize recent advances in our understanding of inhibitors for the TGF-beta superfamily and their medical applications.
Record Date Created: 20061114

2/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

21985750 PMID: 16837207
%Myostatin% inhibition slows muscle atrophy in rodent models of amyotrophic lateral sclerosis.
Holzbaur Erika L F; Howland David S; Weber Nicholas; Wallace Karen; She Yijin; Kwak Seung; Tchistiakova Lioudmilla A; Murphy Erin; Hinson Joseph; Karim Riyez; Tan Xiang Yang; Kelley Pamela; McGill Kevin C; Williams Gareth ; Hobbs Carl; Doherty Patrick; Zaleska Margaret M; Pangalos Menelas N; Walsh Frank S
Department of Physiology, University of Pennsylvania School of Medicine, D400 Richards Building, 3700 Hamilton Walk, Philadelphia, PA 19104-6085, USA. holzbaur@mail.med.upenn.edu
Neurobiology of disease (United States) Sep 2006, 23 (3) p697-707, ISSN 0969-9961--Print Journal Code: 9500169
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease leading to motor neuron cell death, but recent studies suggest that non-neuronal cells may contribute to the pathological mechanisms involved. %Myostatin% is a negative regulator of muscle growth whose function can be inhibited using neutralizing %antibodies% . In this study, we used transgenic mouse and rat models of ALS to test whether treatment with anti-%myostatin% %antibody% slows muscle atrophy, motor neuron loss, or disease

onset and progression. Significant increases in muscle mass and strength were observed in %myostatin%-%antibody%-treated SOD1(G93A) mice and rats prior to disease onset and during early-stage disease. By late stage disease, only diaphragm muscle remained significantly different in treated animals in comparison to untreated controls. %Myostatin% inhibition did not delay disease onset nor extend survival in either the SOD1(G93A) mouse or rat. Together, these results indicate that inhibition of %myostatin% does not protect against the onset and progression of motor neuron degenerative disease. However, the preservation of skeletal muscle during early-stage disease and improved diaphragm morphology and function maintained through late stage disease suggest that anti-%myostatin% therapy may promote some improved muscle function in ALS.
Record Date Created: 20060822
Record Date Completed: 20061101
Date of Electronic Publication: 20060711

2/7/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

21075414 PMID: 16776476
Production of a monoclonal anti-%myostatin% %antibody% and the effects of in ovo administration of the %antibody% on posthatch broiler growth and muscle mass.
Kim Y S; Bobbili N K; Paek K S; Jin H J
Department of Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu 96822, USA. ykim@hawaii.edu
Poultry science (United States) Jun 2006, 85 (6) p1062-71, ISSN 0032-5791--Print Journal Code: 0401150
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
%Myostatin% , a member of the transforming growth factor-beta (TGF-beta) superfamily, is a potent negative regulator of skeletal muscle growth. The objective of this study was to produce a monoclonal anti-%myostatin% %antibody% and to examine the effects of in ovo administration of the %antibody% on posthatch broiler growth and muscle mass. The mature form of %myostatin% was expressed in Escherichia coli and used as an immunogen in producing a monoclonal %antibody% against %myostatin%. One hybridoma clone (mAb-c134) that showed the strongest affinity to the immunogen in Western blot analysis was used in producing a large quantity of monoclonal anti-%myostatin% %antibody%. In Western blot analysis, this %antibody% showed a strong binding affinity to commercially available mature %myostatin% and demonstrated a certain level of cross-reactivity with recombinant human BMP2 but not with recombinant human TGF-beta3 or porcine TGF-beta1. Competitive ELISA demonstrated binding of the %antibody% to the native form of mature %myostatin% in solution. To examine the effects of in ovo administration of the mAb-c134 %antibody%, eggs were injected once with 40 microg of mAb-c134 in 50 mL of PBS either into the albumen or yolk on d 3 of incubation. Controls received no injection. After hatching, chicks were raised for 35 d. Broilers from eggs that had the %antibody% injected into the yolk had significantly heavier body (4.2%) and muscle (5.5%) mass than the controls in both male and female birds. In contrast, no significant effects on body and muscle mass were observed when the mAb-c134 %antibody% was injected into the albumen. The results of this study suggest that immunoneutralization of %myostatin% during embryonic development is a potential means to improve growth potential of broilers.
Record Date Created: 20060616
Record Date Completed: 20060705

2/7/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

21073998 PMID: 16723712
Age-dependent effect of %myostatin% blockade on disease severity in a murine model of limb-girdle muscular dystrophy.
Parsons Stephanie A; Millay Douglas P; Sargent Michelle A; McNally Elizabeth M; Molkentin Jeffery D
Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., ML7020, Cincinnati, OH 45229-3039, USA.
American journal of pathology (United States) Jun 2006, 168 (6) p1975-85, ISSN 0002-9440--Print Journal Code: 0370502
Contract/Grant No.: 5T32 HL07382; HL; NHLBI
Publishing Model Print; Comment in Am J Pathol. 2006 Jun;168(6) 1775-8; Comment in PMID 16723694
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
%Myostatin% (MSTN) is a muscle-specific secreted peptide that functions to limit muscle growth through an autocrine regulatory feedback loop. Loss of MSTN activity in cattle, mice, and humans leads to a profound phenotype of muscle overgrowth, associated with more and larger fibers and enhanced regenerative capacity. Deletion of MSTN in the mdx mouse model of Duchenne muscular dystrophy enhances muscle mass and reduces disease severity. In contrast, loss of MSTN activity in the dyW/dyW mouse model of laminin-deficient congenital muscular dystrophy, a much more severe and lethal disease model, does not improve all aspects of muscle pathology. Here we examined disease severity associated with %myostatin% (mstn-/-) deletion in mice nullizygous for delta-sarcoglycan (scgd-/-), a model of limb-girdle muscular dystrophy. Early loss of MSTN activity achieved either by monoclonal %antibody% administration or by gene deletion each improved muscle mass, regeneration, and reduced fibrosis in scgd-/- mice. However, %antibody% -mediated inhibition of MSTN in late-stage dystrophic scgd-/- mice did not improve disease. These findings suggest that MSTN inhibition may benefit muscular dystrophy when instituted early or if disease is relatively mild but that MSTN inhibition in severely affected or late-stage disease may be ineffective.
Record Date Created: 20060525
Record Date Completed: 20060705

2/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

20665824 PMID: 16436040
The role of %myostatin% and bone morphogenetic proteins in muscular disorders.
Tsuchida Kunihiro
Institute for Comprehensive Medical Science, Division for Therapies against Intractable Diseases, Fujita Health University, Toyoake, Aichi 470-1192, Japan. tsuchida@fujita-hu.ac.jp
Expert opinion on biological therapy (England) Feb 2006, 6 (2) p147-54, ISSN 1744-7682--Electronic Journal Code: 101125414
Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Skeletal muscle is the largest organ in the human body, and plays an important role in body movement and metabolism. Skeletal muscle mass is lost in genetic disorders such as muscular dystrophy, muscle wasting and ageing. Chemicals and proteins that restore muscle mass and function are potential drugs that can improve human health and could be used in the clinic. %Myostatin% is a muscle-specific member of the transforming growth factor (TGF)-beta superfamily that plays an essential role in the negative regulation of muscle growth. Inhibition of %myostatin% activity is a promising therapeutic method for restoring muscle mass and strength. Potential inhibitors of %myostatin% include follistatin domain-containing proteins, %myostatin% propeptide, %myostatin% %antibodies% and chemical compounds. These inhibitors could be beneficial for the development of clinical drugs for the treatment of muscular disorders. Bone morphogenetic protein (BMP) plays a significant role in the development of neuromuscular architecture and its proper functions. Modulation of BMP activity could be

beneficial for muscle function in muscular disorders. This review will describe the current progress in therapy for muscular disorders, emphasising the importance of %myostatin% as a drug target. (59 Refs.)
Record Date Created: 20060126
Record Date Completed: 20060505

2/7/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

20638686 PMID: 16125157
Insulin-like growth factor-1 mediates stretch-induced upregulation of %myostatin% expression in neonatal rat cardiomyocytes.
Shyu Kou-Gi; Ko Wei-Hsu; Yang Wei-Shiung; Wang Bao-Wei; Kuan Peiliang
Department of Education and Research, Shin Kong Wu Ho-Su Memorial Hospital, 95 Wen-Chang Road, Taipei 111, Taiwan. shyukg@ms12.hinet.net
Cardiovascular research (Netherlands) Dec 1 2005, 68 (3) p405-14, ISSN 0008-6363–Print Journal Code: 0077427
Publishing Model Print-Electronic; Comment in Cardiovasc Res. 2005 Dec 1;68(3) 347-9; Comment in PMID 16226233
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
OBJECTIVES: %Myostatin% , a negative regulator of muscle growth, is increased in hypertrophied and infarcted heart. However, the mechanism of regulation is not known. Mechanical stress is an important regulatory factor for cardiomyocyte growth. The aim of the study was to investigate the effect of cyclic stretch on the expression of %myostatin% gene in cardiomyocytes. METHODS: Neonatal Wistar rat cardiomyocytes grown on a flexible membrane base were stretched by vacuum to 20% of maximum elongation at 60 cycles/min. An in vivo model of aorta-caval shunt in adult rats was used to investigate the %myostatin% expression. RESULTS: Cyclic stretch significantly increased %myostatin% protein and mRNA expression after 6 to 18 h of stretch. Addition of the p38 mitogen-activated protein (MAP) kinase inhibitor SB203580, insulin-like growth factor-1 (IGF-1) monoclonal %antibody% , and p38 siRNA 30 min before stretch inhibited the induction of %myostatin% protein. Cyclic stretch increased, while SB203580, IGF-1, and IGF-1 receptor %antibody% abolished, the phosphorylated p38 protein. Gel shift assays showed significant increase of DNA-protein binding activity of myocyte enhancer factor 2 (MEF2) after stretch, and transfection with p38 siRNA abolished the DNA-protein binding activity induced by cyclic stretch. Cyclic stretch significantly increased the IGF-1 secretion from myocytes. Both conditioned media from stretched myocytes and exogenous administration of IGF-1 recombinant protein to the non-stretched myocytes increased %myostatin% protein expression similar to that seen after cyclic stretch. An in vivo model of aorta-caval shunt in adult rats also demonstrated the increased %myostatin% expression in the myocardium. CONCLUSIONS: Cyclic mechanical stretch enhances %myostatin% expression in cultured rat neonatal cardiomyocytes. The stretch-induced %myostatin% is mediated by IGF-1 at least in part through a p38 MAP kinase and MEF2 pathway.
Record Date Created: 20051108
Record Date Completed: 20060503
Date of Electronic Publication: 20050825

2/7/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

19956325 PMID: 16246158
%Myostatin%: a modulator of skeletal-muscle stem cells.
Walsh F S; Celeste A J
Wyeth Research, Collegeville, PA 19426, USA. walshfs@wyeth.com
Biochemical Society transactions (England) Dec 2005, 33 (Pt 6) p1513-7, ISSN 0300-5127–Print Journal Code: 7506897
Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
%Myostatin% , or GDF-8 (growth and differentiation factor-8), was first identified through sequence identity with members of the BMP (bone morphogenetic protein)/TGF-beta (transforming growth factor-beta) superfamily. The skeletal-muscle-specific expression pattern of %myostatin% suggested a role in muscle development. Mice with a targeted deletion of the %myostatin% gene exhibit a hypermuscular phenotype. In addition, inactivating mutations in the %myostatin% gene have been identified in 'double muscled' cattle breeds, such as the Belgian Blue and Piedmontese, as well as in a hypermuscular child. These findings define %myostatin% as a negative regulator of skeletal-muscle development. %Myostatin% binds with high affinity to the receptor serine threonine kinase ActRIIB (activin type IIB receptor), which initiates signalling through a smad2/3-dependent pathway. In an effort to validate %myostatin% as a therapeutic target in a post-embryonic setting, a neutralizing %antibody% was developed by screening for inhibition of %myostatin% binding to ActRIIB. Administration of this antimyostatin %antibody% to adult mice resulted in a significant increase in both muscle mass and functional strength. Importantly, similar results were obtained in a murine model of muscular dystrophy, the mdx mouse. Unlike the %myostatin%-deficient animals, which exhibit both muscle hypertrophy and hyperplasia, the %antibody% -treated mice demonstrate increased musculature through a hypertrophic mechanism. These results validate %myostatin% inhibition as a therapeutic approach to muscle wasting diseases such as muscular dystrophy, sarcopenic frailty of the elderly and amylotrophic lateral sclerosis. (38 Refs.)
Record Date Created: 20051025
Record Date Completed: 20060223

2/7/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

19618409 PMID: 16214131
Insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-5 mediate TGF-beta- and %myostatin%-induced suppression of proliferation in porcine embryonic myogenic cell cultures.
Kamanga-Sollo E; Pampusch M S; White M E; Hathaway M R; Dayton W R
Department of Animal Science, University of Minnesota, 348 Andrew Boss Laboratory, 1354 Eckles Avenue, St. Paul, MN 55108, USA.
Experimental cell research (United States) Nov 15 2005, 311 (1) p167-76, ISSN 0014-4827–Print Journal Code: 0373226
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
We have previously shown that cultured porcine embryonic myogenic cells (PEMC) produce both insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-5 and secrete these proteins into their media. Exogenously added recombinant porcine (rp) IGFBP-3 and rpIGFBP-5 act via IGF-dependent and IGF-independent mechanisms to suppress proliferation of PEMC cultures. Furthermore, immunoneutralization of endogenous IGFBP-3 and IGFBP-5 in the PEMC culture medium results in increased DNA synthesis rate suggesting that endogenous IGFBP-3 and IGFBP-5 suppress PEMC proliferation. TGF-beta superfamily members %myostatin% and TGF-beta1 have also been shown to suppress proliferation of myogenic cells, and treatment of cultured PEMC with either TGF-beta1 or %myostatin% significantly ($P < 0.01$) increases levels of IGFBP-3 and -5 mRNA. We have previously shown that immunoneutralization of IGFBP-3 decreases the proliferation-suppressing activity of TGF-beta1 and %myostatin% . Here, we show that immunoneutralization of IGFBP-5 also significantly ($P < 0.05$) decreases the DNA synthesis-suppressing activity of these molecules. Simultaneous immunoneutralization of both IGFBP-3 and IGFBP-5 in TGF-beta1 or %myostatin% -treated PEMC cultures restores Long-R3-IGF-I-stimulated DNA synthesis rates to 90% of the levels observed in control cultures receiving no TGF-beta1 or %myostatin% treatment ($P < 0.05$). Even though immunoneutralization of IGFBP-3 and -5 increased DNA synthesis rates in

TGF-beta1 or %myostatin% -treated PEMC cultures, phosphosmad2 levels in these cultures were not affected. These findings strongly suggest that IGFBP-3 and IGFBP-5 affect processes downstream from receptor-mediated Smad phosphorylation that facilitate the ability of TGF-beta and %myostatin% to suppress proliferation of PEMC.
Record Date Created: 20051031
Record Date Completed: 20051222
Date of Electronic Publication: 20051006

2/7/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

19559401 PMID: 15791004
%Myostatin% propeptide-mediated amelioration of dystrophic pathophysiology.
Bogdanovich Sasha; Perkins Kelly J; Krag Thomas O B; Whittemore Lisa-Anne ; Khurana Tejvir S
Department of Physiology and Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6085, USA.
FASEB journal - official publication of the Federation of American Societies for Experimental Biology (United States) Apr 2005, 19 (6) p543-9, ISSN 1530-6860--Electronic Journal Code: 8804484
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Mutations in %myostatin% (GDF8) cause marked increases in muscle mass, suggesting that this transforming growth factor-beta (TGF-beta) superfamily member negatively regulates muscle growth. %Myostatin% blockade therefore offers a strategy for reversing muscle wasting in Duchenne's muscular dystrophy (DMD) without resorting to genetic manipulation. Here, we demonstrate that pharmacological blockade using a %myostatin% propeptide stabilized by fusion to IgG-Fc improved pathophysiology of the mdx mouse model of DMD. Functional benefits evidenced by specific force improvement, exceeded those reported previously using %myostatin% %antibody%-mediated blockade. More importantly, use of a propeptide blockade strategy obviates possibilities of anti-idiotypic responses that could potentially limit the effectiveness of %antibody%-mediated %myostatin% blockade strategies over time. This study provides a novel pharmacological strategy for treatment of diseases associated with muscle wasting such as DMD and since it uses an endogenous inhibitor of %myostatin% should help circumvent technical hurdles and toxicity associated with conventional gene or cell based therapies.
Record Date Created: 20050325
Record Date Completed: 20051214

2/7/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

19420503 PMID: 16192645
NS-398, a cyclooxygenase-2-specific inhibitor, delays skeletal muscle healing by decreasing regeneration and promoting fibrosis.
Shen Wei; Li Yong; Tang Ying; Cummins James; Huard Johnny
Departments of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15213, USA.
American journal of pathology (United States) Oct 2005, 167 (4) p1105-17, ISSN 0002-9440--Print Journal Code: 0370502
Contract/Grant No.: 1 R01 AR 47973; AR; NIAMS; C06 RR 14489; RR; NCRR
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Nonsteroidal anti-inflammatory drugs are often prescribed after muscle injury. However, the effect of nonsteroidal anti-inflammatory drugs on muscle healing remains primarily controversial. To further examine the validity of using these drugs after muscle injury, we investigated the working mechanism of NS-398, a cyclooxygenase-2-specific inhibitor. In vitro experiments showed that NS-398 inhibited the proliferation and maturation of differentiated myogenic precursor cells, suggesting a detrimental effect on skeletal muscle healing. Using a mouse laceration model, we analyzed the in vivo effect of NS-398 on skeletal muscle healing at time points up to 4 weeks after injury. The in vivo results revealed delayed muscle regeneration at early time points after injury in the NS-398-treated mice. Compared to controls, lacerated muscles treated with NS-398 expressed higher levels of transforming growth factor-beta1, which corresponded with increased fibrosis. In addition, transforming growth factor-beta1 co-localized with %myostatin% , a negative regulator of skeletal muscle growth. We also found reduced neutrophil and macrophage infiltration in treated muscles, indicating that the delayed skeletal muscle healing observed after NS-398 treatment could be influenced by the anti-inflammatory effect of NS-398. Our findings suggest that the use of cyclooxygenase-2-specific inhibitors to treat skeletal muscle injuries warrants caution because they may interfere with muscle healing.
Record Date Created: 20050929
Record Date Completed: 20051121

2/7/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

15535762 PMID: 15878958
%Myostatin% inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells.
Artaza Jorge N; Bhasin Shalender; Magee Thomas R; Reisz-Porszasz Suzanne; Shen Ruoquin; Groome Nigel P; Fareez Meerasaluh M; Gonzalez-Cadavid Nestor F
Division of Endocrinology, Metabolism, and Molecular Medicine, Charles R. Drew University of Medicine and Science, 1731 East 120th Street, Los Angeles, California 90059, USA. joartaza@cdrewu.edu
Endocrinology (United States) Aug 2005, 146 (8) p3547-57, ISSN 0013-7227--Print Journal Code: 0375040
Contract/Grant No.: 1R01DK59627; DK; NIDDK; 1R01HD043348-01; HD; NICHD; 2R01DK49296-07; DK; NIDDK; 3S06GM068510-02S21; GM; NIGMS; 5P20MD000545-02; MD; NCMHHD; G12RR003026; RR; NCRR; U54HD041748-01; HD; NICHD
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Inactivating mutations of the mammalian %myostatin% gene are associated with increased muscle mass and decreased fat mass; conversely, %myostatin% transgenic mice that overexpress %myostatin% in the skeletal muscle have decreased muscle mass and increased fat mass. We investigated the effects of recombinant %myostatin% protein and antimyostatin %antibody% on myogenic and adipogenic differentiation of mesenchymal multipotent cells. Accordingly, 10T(1/2) cells were incubated with 5'-azacytidine for 3 d to induce differentiation and then treated with a recombinant protein for %myostatin% (Mst) carboxy terminal 113 amino acids or a polyclonal anti-Mst %antibody% for 3, 7, and 14 d. Cells were also cotransfected with a Mst cDNA plasmid expressing the full-length 375-amino acid protein (pcDNA-Mst375) and the silencer RNAs for either Mst (pSil-Mst) or a random sequence (pSil-RS) for 3 or 7 d, and Mst expression was determined. Adipogenesis was evaluated by quantitative image analysis of fat cells before and after oil-red-O staining, immunocytochemistry of adiponectin, and Western blot for CCAAT/enhancer binding protein-alpha. Myogenesis was estimated by quantitative image analysis-immunocytochemistry for MyoD (Myo differentiation protein), myogenin, and myosin heavy chain type II, or by Western blot for myogenin. 5'-Azacytidine-mediated differentiation induced endogenous full-length Mst expression. Recombinant Mst carboxy terminal 113 amino acids inhibited both early and late markers of myogenesis and stimulated both early and late markers of adipogenesis, whereas the %antibody% against Mst exerted the reverse effects. Myogenin levels at 7 d

after transfection of pcDNA-Mst375 were reduced as expected and elevated by pSil-Mst, which blocked efficiently Mst375 expression. In conclusion, %myostatin% promotes the differentiation of multipotent mesenchymal cells into the adipogenic lineage and inhibits myogenesis.
Record Date Created: 20050712
Record Date Completed: 20050926
Date of Electronic Publication: 20050505

2/7/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

15163653 PMID: 15256363
Skeletal muscle atrophy is associated with an increased expression of %myostatin% and impaired satellite cell function in the portacaval anastamosis rat.
Dasarathy Srinivasan; Dodig Milan; Muc Sean M; Kalhan Satish C; McCullough Arthur J
Schwartz Center for Metabolism and Nutrition, Department of Medicine, Division of Gastroenterology, Case Western Reserve University, MetroHealth Medical Center, Cleveland OH 44109, USA. sxd28@case.edu
American journal of physiology. Gastrointestinal and liver physiology (United States) Dec 2004, 287 (6) pG1124-30, ISSN 0193-1857--Print Journal Code: 100901227
Contract/Grant No.: DK-61732; DK; NIDDK
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Proliferation and differentiation of satellite cells are critical in the regeneration of atrophied muscle following immobilization and aging. We hypothesized that impaired satellite cell function is responsible for the atrophy of skeletal muscle also seen in cirrhosis. %Myostatin% and insulin-like growth factor 1 (IGF1) have been identified to be positive and negative regulators, respectively, of satellite cell function. Using a rat model of cirrhosis [portacaval anastamosis (PCA)] and sham-operated controls, we examined the expression of %myostatin% , its receptor activinR2b, and its downstream messenger cyclin-dependent kinase inhibitor p21 (CDKI p21) as well as IGF1 and its receptor in the gastrocnemius muscle. Expression of PCNA, a marker of proliferation, and myogenic regulatory factors (myoD, myf5, and myogenin), markers of differentiation of satellite cells, were also measured. Real- time PCR for mRNA and Western blot assay for protein quantification were performed. PCA rats had lower body weight and gastrocnemius weight compared with sham animals ($P < 0.05$). PCNA and myogenic regulatory factors were lower in PCA rats ($P < 0.05$). %Myostatin% , activinR2b, and CDKI p21 were higher in the PCA animals ($P < 0.05$). The expression of IGF1 and its receptor was lower in liver and skeletal muscle of PCA animals ($P < 0.05$). These data suggest that skeletal muscle atrophy seen in the portacaval shunted rats is a consequence of impaired satellite cell proliferation and differentiation mediated, in part, by higher %myostatin% and lower IGF1 expression.
Record Date Created: 20041109
Record Date Completed: 20041220
Date of Electronic Publication: 20040715

2/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

15111038 PMID: 15470600
Metabolic Diseases Drug Discovery-Strategic Research Institute's Third International World Summit. Dipeptidyl peptidase-IV inhibitors 26-27 July 2004, San Diego, CA, USA.
Xu Jing
BioMinerva Group, 11553 Hadar Drive, San Diego, CA 92126, USA. jxu4@san.rr.com.
IDrugs - the investigational drugs journal (England) Sep 2004, 7 (9) p839-40, ISSN 1369-7056--Print Journal Code: 100883655
Publishing Model Print
Document type: Congresses
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
The majority of the presentations a the conference were on three highly sought-after targets for type 2 diabetes mellitus, namely PTP1B, PPARs and DPP-IV, reflecting the current focus and trend in the industry. A couple of novel targets were discussed, including the potential of %myostatin% as a type 2 diabetes mellitus target and a novel GPCR target. While small molecules were dominant, several biological-based approaches were covered: %antibody% therapeutics and oligonucleotide-based approaches (ASO and siRNA). In searching for small-molecule leads, structure-based rational design and focused combination chemistry appear to produce better results than a random high-throughput approach over the entire chemical library. The biggest challenges for diabetes and obesity drugs remain similar to those mentioned in previous meetings: increasing specificity to reduce side effects and maintaining long-term effect while maintaining or increasing efficacy. Due to the tremendous interest of the pharmaceutical industry in metabolic disease drug development, our knowledge of food intake and metabolism regulation has increased exponentially. Overall, the prospect of better drugs for, and better control of, type 2 diabetes mellitus and obesity is promising.
Record Date Created: 20041007
Record Date Completed: 20050331

2/7/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14930642 PMID: 15192449
Role of %myostatin% in metabolism.
Gonzalez-Cadavid Nestor F; Bhasin Shalender
Division of Endocrinology, Department of Internal Medicine, Charles R. Drew University, Los Angeles, CA, USA. ncadavid@ucla.edu
Current opinion in clinical nutrition and metabolic care (England) Jul 2004, 7 (4) p451-7, ISSN 1363-1950--Print Journal Code: 9804399
Contract/Grant No.: 1R01 AG-14369; AG; NIA; G12RR-03026; RR; NCRR
Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
PURPOSE OF REVIEW: To review papers on %myostatin% published in 2003 and early 2004. %Myostatin% is a negative regulator of skeletal muscle mass produced in this tissue. Inactivating mutations of the %myostatin% gene or interaction of %myostatin% protein with follistatin and other inhibitory proteins induce a hypermuscular phenotype in cattle and mice; this is assumed to result from inhibition of muscle cell proliferation and DNA and protein synthesis (antianabolic effects). %Myostatin% also controls muscle mass in other animals, and appears to affect adipose tissue mass. RECENT FINDINGS: New protein interactions inhibiting %myostatin% that lead to double muscling, as well as the induction of hypermuscularity with %myostatin% %antibodies%, or the generation of a %myostatin% conditional knockout mouse, have been reported. Conversely, a transgenic mouse over-expressing %myostatin% and exhibiting reduced muscle mass in a gender-specific process has been obtained. In addition, novel inactivating mutations in the %myostatin% gene and genetic loci regulating %myostatin% effects, and the characterization of the %myostatin% gene and its effects on metabolism in fish and chicken have been described. Finally, the regulation of %myostatin% levels by growth hormone, glucorticoids, anabolic agents, nutritional status and exercise, the characterization of %myostatin% signaling pathways, and the clarification of %myostatin% effects on cell replication and differentiation, are other important recent findings. SUMMARY: These studies suggest that proteins and drugs that inactivate %myostatin%, or interfere with its binding to its receptor, may be useful for the therapy of wasting and degenerative muscle diseases and for the food industry. Other promising approaches may derive from new

insights into the biochemical cascade that mediates %myostatin% effects, and into the role of %myostatin% in the regulation of fat metabolism and of heart and muscle regeneration after injury. (74 Refs.)
Record Date Created: 20040611
Record Date Completed: 20041202

2/7/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14875989 PMID: 15130492
Deacetylase inhibitors increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin.
Iezzi Simona; Di Padova Monica; Serra Carlo; Caretti Giuseppina; Simone Cristiano; Maklan Eric; Minetti Giulia; Zhao Po; Hoffman Eric P; Puri Pier Lorenzo; Sartorelli Vittorio
Muscle Gene Expression Group, Laboratory of Muscle Biology, NIAMS, National Institutes of Health, Bethesda, MD 20892, USA.
Developmental cell (United States) May 2004, 6 (5) p673-84, ISSN 1534-5807--Print Journal Code: 101120028
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Fusion of undifferentiated myoblasts into multinucleated myotubes is a prerequisite for developmental myogenesis and postnatal muscle growth. We report that deacetylase inhibitors favor the recruitment and fusion of myoblasts into preformed myotubes. Muscle-restricted expression of follistatin is induced by deacetylase inhibitors and mediates myoblast recruitment and fusion into myotubes through a pathway distinct from those utilized by either IGF-1 or IL-4. Blockade of follistatin expression by RNAi-mediated knockdown, functional inactivation with either neutralizing %antibodies% or the antagonist protein %myostatin% , render myoblasts refractory to HDAC inhibitors. Muscles from animals treated with the HDAC inhibitor trichostatin A display increased production of follistatin and enhanced expression of markers of regeneration following muscle injury. These data identify follistatin as a central mediator of the fusigenic effects exerted by deacetylase inhibitors on skeletal muscles and establish a rationale for their use to manipulate skeletal myogenesis and promote muscle regeneration.
Record Date Created: 20040507
Record Date Completed: 20040706

2/7/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14837874 PMID: 15080873
Technologies for the control of fat and lean deposition in livestock.
Sillence M N
School of Agriculture, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. msillence@csu.edu.au
Veterinary journal (London, England - 1997) (England) May 2004, 167 (3) p242-57, ISSN 1090-0233--Print Journal Code: 9706281
Publishing Model Print; Comment in Vet J. 2004 May;167(3) 217-8; Comment in PMID 15080867
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
When the ratio of lean to fat deposition is improved, so is feed conversion efficiency. Net benefits may include lower production costs, better product quality, less excretion of nitrogenous wastes into the environment, decreased grazing pressure on fragile landscapes, and reduced pressure on world feed supplies. However, finding a way to achieve these goals that is reliable, affordable, and acceptable to the majority of consumers has proved to be a major challenge. Since the European Union banned hormonal growth promoters (HGPs) 15 years ago, countries such as Australia and the United States have licensed new products for livestock production, including bovine growth hormone (GH), porcine and equine GH, and the beta-agonist ractopamine. There has also been considerable research into refining these products, as well as developing new technologies. Opportunities to improve beta-agonists include lessening their effects on meat toughness, reducing adverse effects on treated animals, and prolonging their duration of action. In the last regard, the combined use of a beta-agonist with GH, which upregulates beta-adrenoceptors, can produce an outstanding improvement in carcass composition and feed efficiency. Insulin-like growth factor-1 (IGF-1) mediates many of the actions of GH, but has proved to be of more use as a growth reporter/selection marker in pigs, than as a viable treatment. However, a niche for this product could exist in the manipulation of neonatal growth, causing a life-long change in lean:fat ratio. Another significant advance in endocrinology is the discovery of hormones secreted by muscle and fat cells, that regulate feed intake, energy metabolism, and body composition. Leptin, adiponectin and %myostatin% were discovered through the study of genetically obese, or double-muscled animals. Through genetic manipulation, there is potential to exploit these findings in a range of livestock species, although the production of transgenic animals is still hampered by the poor level of control over gene expression, and faces an uphill battle over consumer acceptance. There are several alternatives to HGPs and transgenics, that are more likely to gain world-wide acceptance. Genetic selection can be enhanced by using markers for polymorphic genes that control fat and lean, such as thyroglobulin, or the callipyge gene. Feed additives of natural origin, such as betaine, chromium and conjugated linoleic acid, can improve the fat:lean ratio under specific circumstances. Additionally, 'production vaccines' have been developed, which alter the neuro-endocrine system by causing an auto-immune response. Thus, %antibodies% have been used to neutralise growth-limiting factors, prolong the half-life of anabolic hormones, or activate hormone receptors directly. Unfortunately, none of these technologies is sufficiently well advanced yet to rival the use of exogenous HGPs in terms of efficacy and reliability. Therefore, further research is needed to find ways to control fat and lean deposition with due consideration of industry needs, animal welfare and consumer requirements. (195 Refs.)
Record Date Created: 20040414
Record Date Completed: 20040615

2/7/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14831768 PMID: 15075916
%Myostatin%: a therapeutic target for skeletal muscle wasting.
Roth Stephen M; Walsh Sean
Department of Kinesiology, College of Health and Human Performance, University of Maryland, College Park, Maryland 20742, USA. sroth1@umd.edu
Current opinion in clinical nutrition and metabolic care (England) May 2004, 7 (3) p259-63, ISSN 1363-1950--Print Journal Code: 9804399
Contract/Grant No.: AG022791; AG; NIA
Publishing Model Print
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
PURPOSE OF REVIEW: This review discusses recent developments in %myostatin% research, focusing on the basic actions of %myostatin% on skeletal muscle, the identification of key regulatory elements of the %myostatin% pathway, and the promise of %myostatin% as a therapeutic target in muscle-related disorders. RECENT FINDINGS: In addition to a well-characterized role in muscle development, recent research advances have solidified the importance of %myostatin% in adult muscle, although questions remain. A number of possible regulatory proteins for %myostatin% have been identified, showing a complex picture of %myostatin% regulation that requires clarification. The identification of an antimyostatin monoclonal %antibody% (JA16) shows the promise of %myostatin% as a target for muscle-wasting disorders; the %antibody% has already been shown to

increase muscle mass in healthy older mice and muscle function in postnatal mdx mice. SUMMARY: Since its discovery in 1997, %myostatin% has quickly been established as a key regulator of skeletal muscle mass. Recent developments strengthen the idea that %myostatin% will be an important therapeutic target for muscle-wasting-related disorders, and as more details of %myostatin% regulation and its mechanisms of action are clarified, %myostatin% will continue to dominate the skeletal muscle development and muscle-wasting literature. (54 Refs.)
Record Date Created: 20040412
Record Date Completed: 20040930

2/7/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

14583290 PMID: 14614540
[Functional characterization of recombinant %myostatin% and its inhibitory role to chicken muscle development]
Yang Wei; Wang Kun; Chen Yan; Zhang Yong; Huang Bo; Zhu Da-Hai
Molecular and Cellular Developmental Biology Laboratory, Harbin Institute of Technology, Harbin 150001, China. dahaizhu@hotmail.com
Sheng wu hua xue yu sheng wu wu li xue bao Acta biochimica et biophysica Sinica (China) Nov 2003, 35 (11) p1016-22, ISSN 0582-9879--Print Journal Code: 20730160R
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: CHINESE
Main Citation Owner: NLM
Record type: MEDLINE; Completed
%Myostatin% is a recently discovered member of transforming growth factor beta (TGFbeta) superfamily and shares similar structure features with other members of TGFbeta superfamily. For a better understanding of molecular mechanism of %myostatin% function, the production of C-terminal truncated form of recombinant %myostatin% protein (rMSTN) in E. coli was previously reported. Herein, the functional role of the recombinant %myostatin% in regulating myogenesis in a chicken embryonic myoblasts (CEMs) system was determined. By using flow cytometric analysis, the %myostatin% was found to inhibit cell cycle transition from G1 to S phase and result in a cell cycle arrest at G1. In addition, %myostatin% blocked the multi-nucleus myotube formation and caused a decreased expression of the muscle cell differentiation markers (myogenin and MHC) in CEMs. In this study, a rabbit polyclonal %antibody% against %myostatin% was produced and high affinity and specificity of this anti-%myostatin% %antibody% to recombinant and endogenous %myostatin% were assayed by Western blot analysis. Further studies showed that the %antibody% could also recognize the tissue endogenous %myostatin% of human, mouse and rat. A specific 40 kD band was detected in chicken muscle, which suggested that chicken %myostatin% might have different splicing pattern. Immunofluorescence assay indicated that %myostatin% predominantly existed in the cytosol in C2C12 cells. Taken together, the results show that %myostatin% inhibits chicken muscle cells proliferation and differentiation and down-regulates expression of two differentiation marker gene in CEMs. Remarkably, production of functional recombinant %myostatin% protein and its specific %antibody% provides important reagents for unraveling molecular mechanisms underlying %myostatin% action during myogenesis.
Record Date Created: 20031117
Record Date Completed: 20040309

2/7/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

14583200 PMID: 14614446
Inhibition of autocrine secretion of %myostatin% enhances terminal differentiation in human rhabdomyosarcoma cells.
Ricaud Stephanie; Vernus Barbara; Duclos Michel; Bernardi Henri; Ritvos Olli; Carnac Gilles; Bonnieu Anne
INRA, UMR 866-Differenciation Cellulaire et Croissance, 34060 Montpellier Cedex 1, France.
Oncogene (England) Nov 13 2003, 22 (51) p8221-32, ISSN 0950-9232--Print Journal Code: 8711562
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Rhabdomyosarcomas (RMSs) are one of the most common solid tumor of childhood. Rhabdomyosarcoma (RMS) cells fail to both complete the skeletal muscle differentiation program and irreversibly exit the cell cycle as a consequence of an active repression exerted on the muscle-promoting factor MyoD. %Myostatin% is a negative regulator of normal muscle growth, we have thus studied its possible role in RMS cells. Here, we present evidence that overexpression of %myostatin% is a common feature of RMS since both subtypes of RMS (embryonal RD and alveolar Rh30 cells) express high levels of %myostatin% when compared to nontumoral skeletal muscle cells. Interestingly, we found that inactivation of %myostatin% through overexpression of antisense %myostatin% or of follistatin (a %myostatin% antagonist) constructs enhanced differentiation of RD cells. In addition, RD and Rh30 cells treated with blocking antimyostatin %antibodies% progress into the myogenic terminal differentiation program. Finally, our results suggest that high levels of %myostatin% could impair MyoD function in RMS cells. These results show that an autocrine %myostatin% loop contributes to maintain RMS cells in an undifferentiating stage and suggest that new therapeutic approaches could be exploited for the treatment of RMS based on inactivation of %myostatin% protein.
Record Date Created: 20031117
Record Date Completed: 20040105

2/7/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2006 Dialog. All rts. reserv.

14497508 PMID: 14502562
Role of insulin-like growth factor binding protein (IGFBP)-3 in TGF-beta- and GDF-8 (%myostatin%)-induced suppression of proliferation in porcine embryonic myogenic cell cultures.
Kamanga-Sollo E; Pampusch M S; White M E; Dayton W R
Department of Animal Science, University of Minnesota, St. Paul, Minnesota, USA.
Journal of cellular physiology (United States) Nov 2003, 197 (2) p225-31, ISSN 0021-9541--Print Journal Code: 0050222
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Both transforming growth factor (TGF-beta) and growth and development factor (GDF)-8 (%myostatin%) affect muscle differentiation by suppressing proliferation and differentiation of myogenic cells. In contrast, insulin-like growth factors (IGFs) stimulate both proliferation and differentiation of myogenic cells. In vivo, IGFs are found in association with a family of high-affinity insulin-like growth factor binding proteins (IGFBP 1-6) that affect their biological activity. Treatment of porcine embryonic myogenic cell (PEMC) cultures with either TGF-beta(1) or GDF-8 suppressed proliferation and increased production of IGFBP-3 protein and mRNA ($P < 0.005$). An anti-IGFBP-3 %antibody% that neutralizes the biological activity of IGFBP-3 reduced the ability of either TGF-beta(1) or GDF-8 to suppress PEMC proliferation ($P < 0.005$). However, this %antibody% did not affect proliferation rate in the presence of both TGF-beta(1) and GDF-8. These data show that IGFBP-3 plays a role in mediating the activity of either TGF-beta(1) or GDF-8 alone but not when both TGF-beta(1) and GDF-8 are present. In contrast to findings in T47D breast cancer cells, treatment of PEMC cultures with IGFBP-3 did not result in increased levels of phosphosmad-2. Since TGF-beta and GDF-8 are believed to play a significant role in regulating proliferation and differentiation of myogenic cells, our current data showing that IGFBP-3 plays a role in mediating the activity of these growth factors in muscle cell cultures

strongly suggest that IGFBP-3 also may be involved in regulating these processes in myogenic cells.
Record Date Created: 20030922
Record Date Completed: 20031110

2/7/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14491014 PMID: 12963705
%Myostatin% negatively regulates satellite cell activation and self-renewal.
McCroskery Seumas; Thomas Mark; Maxwell Linda; Sharma Mridula; Kambadur Ravi
Animal Genomics, AgResearch, Hamilton 2015, New Zealand.
Journal of cell biology (United States) Sep 15 2003, 162 (6) p1135-47, ISSN 0021-9525--Print Journal Code: 0375356
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Satellite cells are quiescent muscle stem cells that promote postnatal muscle growth and repair. Here we show that %myostatin%, a TGF-beta member, signals satellite cell quiescence and also negatively regulates satellite cell self-renewal. BrdU labeling in vivo revealed that, among the %Myostatin% -deficient satellite cells, higher numbers of satellite cells are activated as compared with wild type. In contrast, addition of %Myostatin% to myofiber explant cultures inhibits satellite cell activation. Cell cycle analysis confirms that %Myostatin% up-regulated p21, a Cdk inhibitor, and decreased the levels and activity of Cdk2 protein in satellite cells. Hence, %Myostatin% negatively regulates the G1 to S progression and thus maintains the quiescent status of satellite cells. Immunohistochemical analysis with CD34 %antibodies% indicates that there is an increased number of satellite cells per unit length of freshly isolated Mstn-/- muscle fibers. Determination of proliferation rate suggests that this elevation in satellite cell number could be due to increased self-renewal and delayed expression of the differentiation gene (myogenin) in Mstn-/- adult myoblasts. Taken together, these results suggest that %Myostatin% is a potent negative regulator of satellite cell activation and thus signals the quiescence of satellite cells.
Record Date Created: 20030916
Record Date Completed: 20031031
Date of Electronic Publication: 20030908

2/7/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14161468 PMID: 12559968
Inhibition of %myostatin% in adult mice increases skeletal muscle mass and strength.
Whittemore Lisa-Anne; Song Kening; Li Xiangping; Aghajanian Jane; Davies Monique; Girgenrath Stefan; Hill Jennifer J; Jalenak Mary; Kelley Pamela; Knight Andrea; Maylor Rich; O'Hara Denise; Pearson Adele; Quazi Amira; Ryerson Stephanie; Tan Xiang Yang; Tomkinson Kathleen N; Veldman Geertruida M; Widom Angela; Wright Jill F; Wudyka Steve; Zhao Liz; Wolfman Neil M
Musculoskeletal Sciences Department, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, USA. lwhittemore@wyeth.com
Biochemical and biophysical research communications (United States) Jan 24 2003, 300 (4) p965-71, ISSN 0006-291X--Print Journal Code: 0372516
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
A human therapeutic that specifically modulates skeletal muscle growth would potentially provide a benefit for a variety of conditions including sarcopenia, cachexia, and muscular dystrophy. %Myostatin%, a member of the TGF-beta family of growth factors, is a known negative regulator of muscle mass, as mice lacking the %myostatin% gene have increased muscle mass. Thus, an inhibitor of %myostatin% may be useful therapeutically as an anabolic agent for muscle. However, since %myostatin% is expressed in both developing and adult muscles, it is not clear whether it regulates muscle mass during development or in adults. In order to test the hypothesis that %myostatin% regulates muscle mass in adults, we generated an inhibitory %antibody% to %myostatin% and administered it to adult mice. Here we show that mice treated pharmacologically with an %antibody% to %myostatin% have increased skeletal muscle mass and increased grip strength. These data show for the first time that %myostatin% acts postnatally as a negative regulator of skeletal muscle growth and suggest that %myostatin% inhibitors could provide a therapeutic benefit in diseases for which muscle mass is limiting.
Record Date Created: 20030131
Record Date Completed: 20030314

2/7/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14156929 PMID: 12546816
Autoregulation of neurogenesis by GDF11.
Wu Hsiao-Huei; Ivkovic Sanja; Murray Richard C; Jaramillo Sylvia; Lyons Karen M; Johnson Jane E; Calof Anne L
Department of Anatomy and Neurobiology and The Developmental Biology Center, University of California, Irvine, Irvine, CA 92697, USA.
Neuron (United States) Jan 23 2003, 37 (2) p197-207, ISSN 0896-6273 --Print Journal Code: 8809320
Contract/Grant No.: AR44528; AR; NIAMS; DC03583; DC; NIDCD; HD38761; HD; NICHD
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
In the olfactory epithelium (OE), generation of new neurons by neuronal progenitors is inhibited by a signal from neurons themselves. Here we provide evidence that this feedback inhibitory signal is growth and differentiation factor 11 (GDF11). Both GDF11 and its receptors are expressed by OE neurons and progenitors, and GDF11 inhibits OE neurogenesis in vitro by inducing p27(Kip1) and reversible cell cycle arrest in progenitors. Mice lacking functional GDF11 have more progenitors and neurons in the OE, whereas mice lacking follistatin, a GDF11 antagonist, show dramatically decreased neurogenesis. This negative autoregulatory action of GDF11 is strikingly like that of its homolog, GDF8/%myostatin%, in skeletal muscle, suggesting that similar strategies establish and maintain proper cell number during neural and muscular development.
Record Date Created: 20030127
Record Date Completed: 20030404

2/7/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14037248 PMID: 12459784
Functional improvement of dystrophic muscle by %myostatin% blockade.
Bogdanovich Sasha; Krag Thomas O B; Barton Elisabeth R; Morris Linda D; Whittemore Lisa-Anne; Ahima Rexford S; Khurana Tejvir S
Department of Physiology and Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, 3700 Hamilton Walk, Richards A-601, Philadelphia, Pennsylvania 19104-6085, USA.
Nature (England) Nov 28 2002, 420 (6914) p418-21, ISSN 0028-0836--Print Journal Code: 0410462
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: MEDLINE; Completed
Mice and cattle with mutations in the %myostatin% (GDF8) gene show a marked increase in body weight and muscle mass, indicating that this new member of the TGF-beta superfamily is a negative regulator of skeletal muscle growth. Inhibition of the %myostatin% gene product is predicted to increase muscle mass and improve the disease phenotype in a variety of primary and secondary myopathies. We tested the ability of inhibition of %myostatin% in vivo to ameliorate the dystrophic phenotype in the mdx mouse model of Duchenne muscular dystrophy (DMD). Blockade of endogenous %myostatin% by using intraperitoneal injections of blocking %antibodies% for three months resulted in an increase in body weight, muscle mass, muscle size and absolute muscle strength in mdx mouse muscle along with a significant decrease in muscle degeneration and concentrations of serum creatine kinase. The functional improvement of dystrophic muscle by %myostatin% blockade provides a novel, pharmacological strategy for treatment of diseases associated with muscle wasting such as DMD, and circumvents the major problems associated with conventional gene therapy in these disorders.
Record Date Created: 20021202
Record Date Completed: 20021227

2/7/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13984011 PMID: 12194980
The %myostatin% propeptide and the follistatin-related gene are inhibitory binding proteins of %myostatin% in normal serum.
Hill Jennifer J; Davies Monique V; Pearson Adele A; Wang Jack H; Hewick Rodney M; Wolfman Neil M; Qiu Yongchang
Department of Protein Chemistry and Proteomics, Wyeth Research, 87 Cambridge Park Drive, Cambridge, MA 02140, USA. jhill@wyeth.com
Journal of biological chemistry (United States) Oct 25 2002, 277 (43) p40735-41, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
%Myostatin% , also known as growth and differentiation factor 8, is a member of the transforming growth factor beta superfamily that negatively regulates skeletal muscle mass (1). Recent experiments have shown that %myostatin% activity is detected in serum by a reporter gene assay only after activation by acid, suggesting that native %myostatin% circulates as a latent complex (2). We have used a monoclonal %myostatin% %antibody%, JA16, to isolate the native %myostatin% complex from normal mouse and human serum. Analysis by mass spectrometry and Western blot shows that circulating %myostatin% is bound to at least two major proteins, the %myostatin% propeptide and the follistatin-related gene (FLRG). The %myostatin% propeptide is known to bind and inhibit %myostatin% in vitro (3). Here we show that this interaction is relevant in vivo, with a majority (>70%) of %myostatin% in serum bound to its propeptide. Studies with recombinant V5-His-tagged FLRG protein confirm a direct interaction between mature %myostatin% and FLRG. Functional studies show that FLRG inhibits %myostatin% activity in a reporter gene assay. These experiments suggest that the %myostatin% propeptide and FLRG are major negative regulators of %myostatin% in vivo.
Record Date Created: 20021025
Record Date Completed: 20021209
Date of Electronic Publication: 20020822

2/7/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13428673 PMID: 11606186
The two %myostatin% genes of Atlantic salmon (Salmo salar) are expressed in a variety of tissues.
Ostbye T K; Galloway T F; Nielsen C; Gabestad I; Bardal T; Andersen O
Institute of Aquaculture Research, Aas, Norway.
European journal of biochemistry / FEBS (Germany) Oct 2001, 268 (20) p5249-57, ISSN 0014-2956--Print Journal Code: 0107600
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Two %myostatin% isoforms were identified in Atlantic salmon (Salmo salar) by RT-PCR, and genomic sequences encoding this negative muscle growth factor were for the first time isolated from a nonmammalian species. Salmon %myostatin% isoform I is transcribed in white skeletal muscle as a 2346-nucleotide mRNA species that encodes a precursor protein of 373 amino acids. Salmon %myostatin% I shows 93% sequence identity with isoform II which was isolated from white muscle as a partial cDNA sequence of 1409 nucleotides. In contrast to the restricted gene expression of %myostatin% in mammals, salmon %myostatin% I and II mRNAs were identified by RT-PCR in multiple tissues, including white muscle, intestine, brain, gills, tongue and eye. In addition, isoform I mRNA was found in red skeletal muscle, heart, spleen, and ovarian tissue. Using polyclonal %antibodies% against both isoforms, a 55-kDa precursor protein was detected by Western blot analysis in the red and white skeletal muscle, heart, intestine, and brain. Immunoreactive peptides of 35-40 kDa were identified in the gills, tongue, spleen, and head kidney, while the 25-kDa mature %myostatin% was found in the eye and serum, and in vitro expressed in rabbit reticulocyte lysate. Salmon %myostatin% was immunohistochemically localized in the sarcoplasma of red and white muscle fibres, in intestinal epithelial cells, at the basis of the branchial primary lamellae, and in odontoblasts and ameloblasts of the tongue teeth. The results indicate that the role of fish %myostatin% may not be restricted to muscle growth regulation, but may have additional functions similar to the growth/differentiation factor-11 in mammals.
Record Date Created: 20011018
Record Date Completed: 20011207

2/7/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12967929 PMID: 11115768
%Myostatin% and insulin-like growth factor-I and -II expression in the muscle of rats exposed to the microgravity environment of the NeuroLab space shuttle flight.
Lalani R; Bhasin S; Byhower F; Tarnuzzer R; Grant M; Shen R; Asa S; Ezzat S; Gonzalez-Cadavid N F
Division of Endocrinology, Metabolism, and Molecular Medicine, Charles R Drew University, Los Angeles, California 90059, USA.
Journal of endocrinology (ENGLAND) Dec 2000, 167 (3) p417-28, ISSN 0022-0795--Print Journal Code: 0375363
Contract/Grant No.: 1RO1AG14369; AG; NIA; 1RO1DK49296; DK; NIDDK; P20RR11145-01; RR; NCRR; +
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
The mechanism of the loss of skeletal muscle mass that occurs during spaceflight is not well understood. %Myostatin% has been proposed as a negative modulator of muscle mass, and IGF-I and IGF-II are known positive regulators of muscle differentiation and growth. We investigated whether muscle loss associated with spaceflight is accompanied by increased levels of %myostatin% and a reduction in IGF-I and -II levels in the muscle, and whether these changes correlate with an increase in muscle proteolysis and apoptosis. Twelve male adult rats sent on the 17-day NASA STS-90 NeuroLab space flight were divided upon return to earth into two groups, and killed either 1 day later (R1) or after 13 days of acclimatization (R13). Ground-based control rats were maintained for the same periods in either

vivarium (R3 and R15, respectively), or flight-simulated cages (R5 and R17, respectively). RNA and protein were isolated from the tibialis anterior, biceps femoris, quadriceps, and gastrocnemius muscles. %Myostatin%, IGF-I, IGF-II and proteasome 2c mRNA concentrations were determined by reverse transcription/PCR; %myostatin% and ubiquitin mRNA were also measured by Northern blot analysis; %myostatin% protein was estimated by immunohistochemistry; the apoptotic index and the release of 3-methylhistidine were determined respectively by the TUNEL assay and by HPLC. Muscle weights were 19-24% lower in the R1 rats compared with the control R3 and R5 rats, but were not significantly different after the recovery period. The %myostatin%/beta-actin mRNA ratios (means+/-s.e.m.) were higher in the muscles of the R1 rats compared with the control R5 rats: 5.0-fold in tibialis (5.35 +/- 1.85 vs 1.07 +/- 0.26), 3.0-fold in biceps (2.46+/-0.70 vs 0.81 +/- 0.04), 1.9-fold in quadriceps (7.84 +/- 1.73 vs 4.08 +/- 0.52), and 2.2-fold in gastrocnemius (0.99 +/- 0.35 vs 0.44 +/- 0.17). These values also normalized upon acclimatization. Our %antibody% against a %myostatin% peptide was validated by detection of the recombinant human %myostatin% protein on Western blots, which also showed that %myostatin% immunostaining was increased in muscle sections from R1 rats, compared with control R3 rats, and normalized upon acclimatization. In contrast, IGF-II mRNA concentrations in the muscles from R1 rats were 64-89% lower than those in R3 animals. With the exception of the gastrocnemius, IGF-II was also decreased in R5 animals maintained in flight-simulated cages, and normalized upon acclimatization. The intramuscular IGF-I mRNA levels were not significantly different between the spaceflight rats and the controls. No increase was found in the proteolysis markers 3-methyl histidine, ubiquitin mRNA, and proteasome 2C mRNA. In conclusion, the loss of skeletal muscle mass that occurs during spaceflight is associated with increased %myostatin% mRNA and protein levels in the skeletal muscle, and a decrease in IGF-II mRNA levels. These alterations are normalized upon restoration of normal gravity and caging conditions. These data suggest that reciprocal changes in the expression of %myostatin% and IGF-II may contribute to the multifactorial pathophysiology of muscle atrophy that occurs during spaceflight.
Record Date Created: 20010130
Record Date Completed: 20011004

2/7/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12421319 PMID: 10362012
%Myostatin%, a transforming growth factor-beta superfamily member, is expressed in heart muscle and is upregulated in cardiomyocytes after infarct.
Sharma M; Kambadur R; Matthews K G; Somers W G; Devlin G P; Conaglen J V; Fowke P J; Bass J J
Growth Physiology, AgResearch, Ruakura, Hamilton, New Zealand. SharmaM@agresearch.cri.nz
Journal of cellular physiology (UNITED STATES) Jul 1999, 180 (1) p1-9, ISSN 0021-9541--Print Journal Code: 0050222
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
%Myostatin% is a secreted growth and differentiating factor (GDF-8) that belongs to the transforming growth factor-beta (TGF-beta) superfamily. Targeted disruption of the %myostatin% gene in mice and a mutation in the third exon of the %myostatin% gene in double-muscled Belgian Blue cattle breed result in skeletal muscle hyperplasia. Hence, %myostatin% has been shown to be involved in the regulation of skeletal muscle mass in both mice and cattle. Previous published reports utilizing Northern hybridization had shown that %myostatin% expression was seen exclusively in skeletal muscle. A significantly lower level of %myostatin% mRNA was also reported in adipose tissue. Using a sensitive reverse transcription-polymerase chain reaction (RT-PCR) technique and Western blotting with anti-%myostatin% %antibodies%, we show that %myostatin% mRNA and protein are not restricted to skeletal muscle. We also show that %myostatin% expression is detected in the muscle of both fetal and adult hearts. Sequence analysis reveals that the Belgian Blue heart %myostatin% cDNA sequence contains an 11 nucleotide deletion in the third exon that causes a frameshift that eliminates virtually all of the mature, active region of the protein. Anti-%myostatin% immunostaining on heart sections also demonstrates that %myostatin% protein is localized in Purkinje fibers and cardiomyocytes in heart tissue. Furthermore, following myocardial infarction, %myostatin% expression is upregulated in the cardiomyocytes surrounding the infarct area. Given that %myostatin% is expressed in fetal and adult hearts and that %myostatin% expression is upregulated in cardiomyocytes after the infarction, %myostatin% could play an important role in cardiac development and physiology.
Record Date Created: 19990617
Record Date Completed: 19990617

2/7/29 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0016315990 BIOSIS NO.: 200600661385
Method for down-regulating GDF-8 activity using immunogenic GDF-8 analogues
AUTHOR: Anonymous; Halkier Torben; Mouritsen Soren; Klysner Steen
AUTHOR ADDRESS: Solrod Strand, Denmark**Denmark
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents JUL 4 2006 2006
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Disclosed are novel methods for increasing muscle mass by means of immunization against Growth Differentiation Factor 8 (GDF-8, %myostatin%). Immunization is preferably effected by administration of analogues of GDF-8 which are capable of inducing %antibody% production against homologous GDF-8. Especially preferred as an immunogen is homologous GDF-8 which has been modified by introduction of one single or a few foreign, immunodominant and promiscuous T-cell epitopes while substantially preserving the tertiary structure of the homologous GDF-8. Also disclosed are nucleic acid vaccination against GDF-8 and vaccination using live vaccines as well as methods and means useful for the vaccination. Such methods and means include methods for identification of useful immunogenic GDF-8 analogues, methods for the preparation of analogues and pharmaceutical formulations, as well as nucleic acid fragments, vectors, transformed cells, polypeptides and pharmaceutical formulations.

2/7/30 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0016296898 BIOSIS NO.: 200600642293
Method for down-regulating GDF-8 activity
AUTHOR: Anonymous; Klysner Steen; Mouritsen Soren; Halkier Torben
AUTHOR ADDRESS: Hillerod, Denmark**Denmark
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents JUN 6 2006 2006
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Disclosed are novel methods for increasing muscle mass by means of immunization against Growth Differentiation Factor 8 (GDF-8, %myostatin%). Immunization is preferably effected by administration of analogues of GDF-8 which are capable of inducing %antibody% production against homologous GDF-8. Especially preferred as an immunogen is homologous GDF-8 which has been modified by introduction of one single or a few foreign, immunodominant and promiscuous T-cell epitopes while

substantially preserving the tertiary structure of the homologous GDF-8. Also disclosed are nucleic acid vaccination against GDF-8 and vaccination using live vaccines as well as methods and means useful for the vaccination. Such methods and means include methods for identification of useful immunogenic GDF-8 analogues, methods for the preparation of analogues and pharmaceutical formulations, as well as nucleic acid fragments, vectors, transformed cells, polypeptides and pharmaceutical formulations.

2/7/31 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0016085753 BIOSIS NO.: 200600431148
Does blood flow restriction enhance hypertrophic signaling in skeletal muscle?
AUTHOR: Meyer Ronald A (Reprint)
AUTHOR ADDRESS: Michigan State Univ, Dept Physiol, E Lansing, MI 48824 USA **USA
AUTHOR E-MAIL ADDRESS: meyerr@msu.edu
JOURNAL: Journal of Applied Physiology 100 (5): p1443-1444 MAY 2006 2006
ISSN: 8750-7587
DOCUMENT TYPE: Article; Editorial
RECORD TYPE: Citation
LANGUAGE: English

2/7/32 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015999430 BIOSIS NO.: 200600344825
Purification and characterization of recombinant activin receptor type IIB kinase expressed in Sf9 cells
AUTHOR: Tam Amy Sze Pui (Reprint); Bean Kevin; Kelleher Kerry; Marvell Todd ; Ross Cindy; Kim Richard; Celeste Tony; Siege Marshall; Kriz Ron; Somers Will; Stahl Mark; Lin Laura
AUTHOR ADDRESS: Wyeth Res, Dept Chem and Screening Sci, Cambridge, MA 02140 USA**USA
JOURNAL: FASEB Journal 20 (4, Part 1): pA498 MAR 6 2006 2006
CONFERENCE/MEETING: Experimental Biology 2006 Meeting San Francisco, CA, USA April 01 -05, 2006; 20060401
SPONSOR: Amer Assoc Anatomists
Amer Physiol Soc
Amer Soc Biochem & Mol Biol
Amer Soc Investigat Pathol
Amer Soc Nutr
Amer Soc Pharmacol & Expt Therapeut
ISSN: 0892-6638
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Activin Receptor Type IIB (ActRIIB) is a member of the transforming growth factor-beta 1 (TGF-beta 1) superfamily that has been shown to bind to %myostatin%/GDF-8. Upon binding to GDF-8, ActRIIB receptor recruits the type I receptor ALK4/5, phosphorylates ALK4/5 GS-rich region and leads to phosphorylation and activation of Smad proteins, which then translocate to the nucleus to regulate gene expression. In mice with dominant negative ActRIIB, they exhibited increase muscle mass and muscle tissue but no phenotypcial abnormality. Disruption of binding between GDF-8 and ActRIIB receptor by administration of GDF-8 neutralizing %antibody% in mice also showed an increase of muscle tissue and muscle strength.These results validate ActRIIB receptor as a good target for small molecule inhibitors for treatment of diseases such as muscular dystrophy. To aide in our structure-based drug design, we have successfully cloned and expressed numerous constructs of ActRIIB kinase domain using the baculovirus expression system. We were able to achieve >= 95% purity of ActRIIB kinase and biochemical characterizations such as activity and phosphorylation state were used to evaluate the different constructs.

2/7/33 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015619124 BIOSIS NO.: 200510313624
Insulin-like growth factor-1 mediates stretch-induced upregulation of %myostatin% expression via p38 MAP kinase and MEF2 pathway in cultured rat neonatal cardiomyocytes
AUTHOR: Wang Bao-Wei (Reprint); Shyu Kou-Gi; Kuan Peiliang; Yang Wei-Shiung
AUTHOR ADDRESS: Mem Hosp, Taipei, Taiwan**Taiwan
JOURNAL: Circulation 110 (17, Suppl. S): p10 OCT 26 2004 2004
CONFERENCE/MEETING: 77th Scientific Meeting of the American-Heart-Association New Orleans, LA, USA November 07 -10, 2004; 20041107
SPONSOR: Amer Heart Assoc
ISSN: 0009-7322
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background. Myostain, a negative regulator of muscle growth, is increased in hypertrophied and infarcted heart, However, the mechanism of regulation is not known. Mechanical stress is an important regulatory factor for cardiomyocyte growth. We sought to investigate the effect of cyclical stretch on the expression of %myostatin% gene in cardiac myocytes and investigate the possible mechanism and signal pathways mediating the expression of %myostatin% gene by cyclical mechanical stretch. Methods and results; Neonatal Wistar rat cardiac myocytes grown on a flexible membrane base were stretched by vacuum to 20% of maximum elongation, at 60 cycles/min. The levels of %myostatin% protein began to increase as early as 6 h after stretch at 20% elongation was applied, reached a maximum of 4.2-fold (P<0.01) over the control by 18 h and remained elevated up to 24 h. The Northern blots showed that %myostatin% messages increased significantly after 6 h and 18 In of stretch at 20% elongation. Cyclical stretch also increased the immunohistochemical labeling of %myostatin%. Gel shifting assay showed that cyclical stretch of myocytess for 2 to 24 h significantly increased the DNA-protein binding activity of MEF2. Addition of SB203580 and IGF-1 monoclonal %antibody% 30 min before stretch and transfection with p38 siRNA inhibited the induction of %myostatin% protein, abolished the DNA-protein binding activity, and decreased immunohistochemical labeling of %myostatin% induced by cyclical stretch. However, the %myostatin% protein induced by stretch was not affected by the addition of PD98059 (50 mu M) or SP600125 (20 mu M). Cyclical stretch significantly began to increase the IGF-1 secretion from myocytes at 2 h after stretch and reached a maximum at 6 h and remained elevated for 24 h. Both conditioned media from stretched myocytes and exogenous administration of IGF-1 recombinant protein to the non-stretched myocytes increased %myostatin% protein expression similar to that seen after cyclical stretch. Conclusions: Cyclical mechanical stretch enhances %myostatin% expression in cultured neonatal cardiac myocytes. The stretch-induced %myostatin% is mediated by IGF-1 at least in part, through p38 MAP kinase and MEF2 pathway.

2/7/34 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015458778 BIOSIS NO.: 200510153278
%Myostatin% is accumulated and complexes with amyloid-beta within muscle fibers of inclusion-body myositis (s-IBM)
AUTHOR: Wojcik Slawomir (Reprint); Engel W King; McFerrin Janis; Askanas Valerie
JOURNAL: Neurology 64 (6, Suppl. 1): pA158 MAR 22 05 2005
CONFERENCE/MEETING: 57th Annual Meeting of the American-Academy-of-Neurology Miami Beach, FL, USA April 09 -19, 2005;

20050409
SPONSOR: Amer Acad Neurol
ISSN: 0028-3878
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/35 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015228274 BIOSIS NO.: 200500134911
Loss of %myostatin% expression alters fiber-type distribution and expression of myosin heavy chain isoforms in slow- and fast-type skeletal muscle
AUTHOR: Girgenrath Stefan (Reprint); Song Kening; Whittemore Lisa-Anne
AUTHOR ADDRESS: Wyeth Dept Cardiovasc and Metab Dis, 87 Cambridge Pk Dr, Cambridge, MA, 02140, USA**USA
AUTHOR E-MAIL ADDRESS: sgirgenrath@netcape.net
JOURNAL: Muscle & Nerve 31 (1): p34-40 January 2005 2005
MEDIUM: print
ISSN: 0148-639X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %Myostatin% (Mstn) is a member of the transforming growth factor-beta family that negatively regulates skeletal muscle mass. Mstn knockout mice have greater skeletal muscle mass than wild-type littermates. We investigated the effect of Mstn on fiber type by comparing adult muscles from the murine Mstn knockout with wild-type controls. Based on myofibrillar ATPase staining, the soleus of Mstn knockout mice displays a larger proportion of fast type II fibers and a reduced proportion of slow type I fibers compared with wild-type animals. Based on staining for succinate dehydrogenase (SDH) activity, a larger proportion of glycolytic fibers and a reduced proportion of oxidative fibers occur in the extensor digitorum longus (EDL) of Mstn knockouts. These differences in distribution of fiber types are accompanied by differences in the expression of myosin heavy chain (MHC) isoforms. In both Mstn knockout soleus and EDL, larger numbers of faster MHC isoforms are expressed at the expense of slower isoforms when compared with wild-type littermates. Thus, the absence of Mstn in the knockout mouse leads to an overall faster and more glycolytic muscle phenotype. This muscle phenotype is likely a consequence of developmental processes, and inhibition of Mstn in adults does not cause a transformation to a more fast and glycolytic phenotype. Our findings suggest that %myostatin% has a critical role in regulating the formation, proliferation, or differentiation of fetal myoblasts and postnatal fibers.

2/7/36 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015067195 BIOSIS NO.: 200400447984
Pneumatic resistance machines can provide eccentric loading - Response
AUTHOR: Willoughby Darryn (Reprint)
AUTHOR ADDRESS: Dept Hlth Human Performance and Recreat, Baylor Univ, Waco, TX, USA**USA
JOURNAL: Medicine & Science in Sports & Exercise 36 (9): p1656 September 2004 2004
MEDIUM: print
ISSN: 0195-9131
DOCUMENT TYPE: Letter; Editorial
RECORD TYPE: Citation
LANGUAGE: English

2/7/37 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0015067194 BIOSIS NO.: 200400447983
Pneumatic resistance machines can provide eccentric loading
AUTHOR: Roth Stephen M (Reprint); Rogers Marc A; Hurley Ben F; Martel Gregory F
AUTHOR ADDRESS: Dept KinesiolColl Hlth and Human Performance, Univ Maryland, College Pk, MD, USA**USA
JOURNAL: Medicine & Science in Sports & Exercise 36 (9): p1655 September 2004 2004
MEDIUM: print
ISSN: 0195-9131
DOCUMENT TYPE: Letter; Editorial
RECORD TYPE: Citation
LANGUAGE: English

2/7/38 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0014442885 BIOSIS NO.: 200300401604
%Myostatin% expression after resistance exercise in younger and older men and women.
AUTHOR: Bamman Marcas M (Reprint); Ragan Ron C; Cross James M
AUTHOR ADDRESS: Physiology and Biophysics, University of Alabama at Birmingham, VAMC GRECC/11G, 1530 3rd Avenue South, Birmingham, AL, 35294-0001, USA**USA
AUTHOR E-MAIL ADDRESS: mbamman@uab.edu; ronald.ragan2@med.va.gov; james.cross@ccc.uab.edu
JOURNAL: FASEB Journal 17 (4-5): pAbstract No. 318.18 March 2003 2003
MEDIUM: e-file
CONFERENCE/MEETING: FASEB Meeting on Experimental Biology: Translating the Genome San Diego, CA, USA April 11-15, 2003; 20030411
SPONSOR: FASEB
ISSN: 0892-6638 _(ISSN print)
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %Myostatin% inhibits myoblast proliferation and thus limits muscle development by regulating myofiber number. In differentiated adult skeletal muscle, load-mediated hypertrophy involves activation of myoblast precursor (i.e. satellite) cells. Whether %myostatin% influences satellite cell activity is not clear. We tested the hypothesis that %myostatin% protein expression would decrease following resistance exercise (RE). We also evaluated the influence of age or gender. Eleven younger (Y; 27 +- 1 yr, 169 +- 3 cm, 68 +- 4 kg) and 12 older (O; 65 +- 1 yr, 171 +- 4 cm, 76 +- 5 kg) adults underwent vastus lateralis biopsy before and 24 h after RE including 3 x 8-12RM of squat, leg press, and knee extension. Twelve women (W; 7 Y, 5 O) and 11 men (M; 4 Y, 7 O) participated. Relative expression of %myostatin% was determined by immunoblotting using an %antibody% against N-terminus of the %myostatin% precursor, yielding the 37 kDa latency-associated peptide after proteolytic processing. Results were analyzed by age or gender x time repeated measures ANOVA. %Myostatin% levels tended to decrease in M (-18%, p=0.06) but not in W after RE. Although not significant, relative changes in %myostatin% after RE by group were -14% (YM), -24% (OM), -17% (YW), and +18% (OW). We conclude that skeletal muscle %myostatin% levels are highly variable after acute RE loading. Although further study is required, there may be a decline in M and YW that is not seen in OW.

2/7/39 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0013988184 BIOSIS NO.: 200200581695
Polyclonal %antibodies% recognize only the latent peptide of %myostatin%

but not the active form of %myostatin% in the chicken
AUTHOR: Kim Y S (Reprint); Lee Y K (Reprint); Dunn M A (Reprint)
AUTHOR ADDRESS: University of Hawaii at Manoa, Honolulu, HI, USA**USA
JOURNAL: Journal of Dairy Science 85 (Supplement 1): p211-212 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Meeting of the American Society of Animal Science and the American Dairy Science Association Quebec City, Quebec, Canada July 20-25, 2002; 20020720
SPONSOR: American Society of Animal Science
American Dairy Science Association
ISSN: 0022-0302
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/40 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0013988183 BIOSIS NO.: 200200581694
Insulin-like growth factor binding protein (IGFBP)-3 is partially responsible for the proliferation-suppressing activity of transforming growth factor beta (TGF beta) on porcine embryonic myogenic cell cultures
AUTHOR: Kamanga-Sollo E I (Reprint); Pampusch M S (Reprint); White M E (Reprint); Hathaway M R (Reprint); Dayton W R (Reprint)
AUTHOR ADDRESS: University of Minnesota, St. Paul, MN, USA**USA
JOURNAL: Journal of Dairy Science 85 (Supplement 1): p211 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Meeting of the American Society of Animal Science and the American Dairy Science Association Quebec City, Quebec, Canada July 20-25, 2002; 20020720
SPONSOR: American Society of Animal Science
American Dairy Science Association
ISSN: 0022-0302
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/41 (Item 13 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0013902997 BIOSIS NO.: 200200496508
Molecular cloning of equine (Thoroughbred) %myostatin% cDNA and detection of %myostatin% precursor proteins in the serum
AUTHOR: Hosoyama Tohru; Kawada Shigeo; Oshiumi Ryosuke; Yoneda Sachie; Soeta Chie; Yamanouchi Keitaro; Hasegawa Telhisa; Ishida Nobushige; Mukoyama Harutaka; Ishii Naokata; Tachi Chikashi (Reprint)
AUTHOR ADDRESS: Laboratory of Developmental and Reproductive Biotechnology, Department of Animal Resource Sciences, School of Veterinary Medicine and Life Sciences, Azabu University, Sagamihara-shi, 229-8501, Japan**Japan
JOURNAL: Journal of Reproduction and Development 48 (4): p335-342 August, 2002 2002
MEDIUM: print
ISSN: 0916-8818
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Growth/Differentiation Factor 8, or GDF-8, is a member of the TGF-beta family and is expressed specifically in myogenic precursor cells of the myotome during development as well as in adult axial and paraxial muscles of the mouse. It acts as a negative regulator of skeletal muscle mass, and is known as %myostatin%. As a part of our studies to elucidate mechanisms underlying the growth of skeletal muscle mass due to physical training in mammals including horses and humans, we isolated and cloned %myostatin% cDNA from equine (Thoroughbred) skeletal muscles by an RT-PCR method. The base sequence of the entire coding region of equine %myostatin% cDNA (DDBJ accession no. AB033541) exhibited a high degree of homology to those of other species so far reported. In the deduced amino acid sequence, 4 amino acid positions were unique to the equine %myostatin%, i. e., Val15 instead of Met, Leu18 instead of Val or Ala, Ala201 instead of Thr, Arg244 instead of Gly or Glu in other species. Western blot analysis of the equine skeletal muscles using anti-%myostatin% %antibody%, yielded 3 immunoreactive bands, i. e., 26, 45 and 50 kDa. The band at approximately 26 kDa probably represents mature %myostatin% and another at approximately 50 kDa corresponds in size to the unprocessed precursor %myostatin% proteins reported in the literature. Western blot analysis of equine sera of 2 independent breeds, Thoroughbred and Kiso-uma, revealed the possible presence of precursor %myostatin% proteins at relatively high levels. The molecular identity and the biological significance of serum %myostatin% immunoreactivities remain to be clarified.

2/7/42 (Item 14 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0013484801 BIOSIS NO.: 200200078312
Comparison of contractile characteristics of muscle from Holstein and double-muscled Belgian Blue foetuses
AUTHOR: Deveaux V; Cassar-Malek I; Picard B (Reprint)
AUTHOR ADDRESS: Unite de Recherches sur les Herbivores, Equipe Croissance et Metabolismes du Muscle, INRA, INRA Clermont-Ferrand/Theix, 63122, Saint-Genes-Champanelle, France**France
JOURNAL: Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology 131A (1): p21-29 December, 2001 2001
MEDIUM: print
ISSN: 1095-6433
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The aim of the present study was to precise the origin of the particular muscle characteristics of double-muscled cattle by comparing muscle properties of Holstein and double-muscled Belgian Blue (BB) foetuses. Ten 100-day-old foetuses of each genotype were studied. The weight and length of foetuses and the length, weight and area of the Semitendinosus (ST) muscle were analysed. Contractile differentiation of the different fibre types was studied by immunohistochemistry using several monoclonal %antibodies% raised against different myosin heavy chain isoforms (MHC slow, fast, foetal) and by electrophoresis. Proliferation phase of myoblasts from each genotype was analysed in primary culture. On 100 days of foetal life, the foetuses of both genotypes did not show any significant differences in their weight and length. However, BB cattle already present muscle hypertrophy, which seems to originate from a higher myoblast proliferation observed in primary culture. The use of anti-MHC %antibodies% shows that ST muscle of BB contained a smaller proportion of primary fibres and a higher proportion of secondary fibres which will give principally fast fibres in adult muscle. Electrophoresis analysis confirms a lower proportion of slow MHC in ST of BB.

2/7/43 (Item 15 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0011920840 BIOSIS NO.: 199900180500
Porcine %myostatin% in muscle and mammary gland development
AUTHOR: Ji S; Willis G M; Cornelius S G; Frank G R; Spurlock M E
AUTHOR ADDRESS: Purina Mills Inc., St. Louis, MO 63144, USA**USA
JOURNAL: FASEB Journal 13 (4 PART 1): pA415 March 12, 1999 1999
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Professional Research Scientists for Experimental Biology 99 Washington, D.C., USA April 17-21, 1999; 19990417

ISSN: 0892-6638
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

2/7/44 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

14130866 EMBASE No: 2006549556
Cachexia: Pathophysiology and clinical relevance
Morley J.E.; Thomas D.R.; Wilson M.-M.G.
J.E. Morley, Division of Geriatric Medicine, Saint Louis University, School of Medicine, 1402 South Grand Boulevard, M238, St Louis, MO 63104 United States
AUTHOR EMAIL: morley@slu.edu
American Journal of Clinical Nutrition (AM. J. CLIN. NUTR.) (United States) 01 APR 2006, 83/4 (735-743)
CODEN: AJCNA ISSN: 0002-9165
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 143

Cachexia causes weight loss and increased mortality. It affects more than 5 million persons in the United States. Other causes of weight loss include anorexia, sarcopenia, and dehydration. The pathophysiology of cachexia is reviewed in this article. The major cause appears to be cytokine excess. Other potential mediators include testosterone and insulin-like growth factor I deficiency, excess %myostatin%, and excess glucocorticoids. Numerous diseases can result in cachexia, each by a slightly different mechanism. Both nutritional support and orexigenic agents play a role in the management of cachexia. (c) 2006 American Society for Nutrition.

2/7/45 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13931892 EMBASE No: 2006328251
%Myostatin%: Biology and clinical relevance
Carnac G.; Ricaud S.; Vernus B.; Bonnieu A.
A. Bonnieu, INRA, UMR 866-Differenciation Cellulaire et Croissance, 34060 Montpellier Cedex 1 France
AUTHOR EMAIL: bonnieu@ensam.inra.fr
Mini-Reviews in Medicinal Chemistry (MINI-REV. MED. CHEM.) (Netherlands) 2006, 6/7 (765-770)
CODEN: MMCIA ISSN: 1389-5575
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 56

%Myostatin% is a negative regulator of muscle mass. Important advances in our understanding of the complex biology of this factor have revealed the therapeutic potential of antagonizing the %myostatin% pathway. Here we present the rationale for evaluating anti-%myostatin% therapies in human muscle-wasting disorders. (c) 2006 Bentham Science Publishers Ltd.

2/7/46 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13900351 EMBASE No: 2006315615
Metabolic and immunologic derangements in cardiac cachexia: Where to from here?
Strassburg S.; Anker S.D.
S.D. Anker, Applied Cachexia Research, Dept. of Cardiology, Charite, Campus Virchow-Klinikum, Berlin Germany
AUTHOR EMAIL: s.anker@cachexia.de
Heart Failure Reviews (HEART FAIL. REV.) (Netherlands) 2006, 11/1 (57-64)
CODEN: HFREF ISSN: 1382-4147
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 105

The onset of cardiac cachexia is characterized by a defined severe weight loss in patients with advanced chronic heart failure and it predicts an increased mortality in these patients. Recent studies with potential therapeutics investigated the effects and efficiency of beta-blockers, ghrelin, or ghrelin-agonists in cachexia. These and other new studies, like the influence of heart transplantation on cardiac cachexia, give prospect into potential therapeutic options in the future. General aim of the treatment strategy is to prevent the onset and retard the progress of cachexia. This could be achieved by modifying the metabolic, neurohormonal and immune system abnormalities, e.g. with beta-blockers and angiotensin-converting enzyme inhibitors. However, these alterations interact in a complex pathophysiological process, which is supposed to end in a vicious circle and thereby the wasting process is further promoted. To interrupt this, an early start of therapy is important to decelerate the development of cardiac cachexia. Many further investigations are needed to find out more about the pathophysiological pathways, to confirm the previous results, and to evaluate new therapeutics. (c) Springer Science + Business Media, LLC 2006.

2/7/47 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13766567 EMBASE No: 2006192256
TGFbeta in cancer and other diseases - AACR special conference in cancer research. 8-12 February 2006, La Jolla, CA, USA
Jachimczak P.
P. Jachimczak, Antisense Pharma GmbH, Josef-Engert-Strasse 9, D-93053 Regensburg Germany
AUTHOR EMAIL: jachimczak@t-online.de
IDrugs (IDRUGS) (United Kingdom) 2006, 9/4 (239-241)
CODEN: IDRUF ISSN: 1369-7056
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The various research approaches with TGFbeta that were presented at the AACR meeting highlight the significant role of TGFbeta signaling on tumor cells and their associated microenvironment. Because of the pivotal role of TGFbeta on malignancy progression, the targeted inhibition of TGFbeta activity may result in beneficial clinical responses, particularly in advanced stages of cancer. An optimal dosing schedule remains a major issue regarding the clinical development of anti-TGFbeta therapy. TGFbeta inhibition must occur at a level that is sufficient to achieve therapeutic effects while avoiding potential toxicity effects due to systemic inactivation. TGFbeta inhibitors may revolutionize the fields of tumor immunotherapy and other diseases, such as fibrotic disorders, for which the overexpression of TGFbeta has a strong pathogenetic effect. (c) The Thomson Corporation.

2/7/48 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13508799 EMBASE No: 2006002332
Society for Neuroscience - 35th Annual Meeting. Stroke, amyotrophic lateral sclerosis and epilepsy. 12-16 November 2005, Washington, DC, USA
Tear S.; Shah S.
S. Tear, Thomson Scientific, 34-42 Cleveland Street, London W1T 4JE United Kingdom
AUTHOR EMAIL: steven.tear@thomson.com
IDrugs (IDRUGS) (United Kingdom) 2005, 8/12 (966-967)

CODEN: IDRUF ISSN: 1369-7056
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: ENGLISH

2/7/49 (Item 6 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13437633 EMBASE No: 2005517985
Cancer cachexia
Gordon J.N.; Green S.R.; Goggin P.M.
Dr. J.N. Gordon, Southampton General Hospital, Mailpoint 813, Southampton SO16 6YD United Kingdom
AUTHOR EMAIL: j.gordon@soton.ac.uk
QJM - Monthly Journal of the Association of Physicians (QJM MON. J. ASSOC. PHYS.) (United Kingdom) 2005, 98/11 (779-788)
CODEN: QMJPF ISSN: 1460-2725
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 83

Cancer cachexia is a severe debilitating disorder for which there are currently few therapeutic options. It is driven by the release of pro-inflammatory cytokines and cachectic factors by both host and tumour. Over the past few years, basic science advances have begun to reveal the breadth and complexity of the immunological mechanisms involved, and in the process have uncovered some novel potential therapeutic targets. The effectiveness of thalidomide and eicosapentaenoic acid at attenuating weight loss in clinical trials also provides a further rationale for modulating the immune response. We are now entering an exciting period in cachexia research, and it is likely that the next few years will see effective new biological therapies reach clinical practice. (c) The Author 2005. Published by Oxford University Press on behalf of the Association of Physicians. All rights reserved.

2/7/50 (Item 7 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13436258 EMBASE No: 2005490687
Muscle regeneration through %myostatin% inhibition
Wagner K.R.
Dr. K.R. Wagner, Department of Neurology, Johns Hopkins School of Medicine, Meyer 5-119, 600 N. Wolfe Street, Baltimore, MD 21287 United States
AUTHOR EMAIL: kwagner@jhmi.edu
Current Opinion in Rheumatology (CURR. OPIN. RHEUMATOL.) (United States) 2005, 17/6 (720-724)
CODEN: CORHE ISSN: 1040-8711
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 36

Purpose of review: %Myostatin% is an endogenous, negative regulator of muscle growth. Selective inhibition of %myostatin% may have broad clinical utility by improving regeneration in diverse and burdensome muscle disorders. An understanding of this potential is relevant because inhibitors of %myostatin% have recently entered clinical trials. Recent findings: This article reviews the structure and function of %myostatin%, the effect of inhibiting %myostatin% in models of disease, and potential therapeutic approaches to blocking %myostatin% pharmacologically. The possibility that a %myostatin% inhibitor will promote muscle regeneration in human disease, as seen in animal models, is suggested by the observation that loss of %myostatin% results in muscle hypertrophy in a human subject. Summary: Multiple approaches to inhibiting %myostatin% are suggested by the recent elucidation of its signaling pathway. An inhibitor of %myostatin% may be the first drug specifically designed to enhance muscle growth and regeneration. (c) 2005 Lippincott Williams & Wilkins.

2/7/51 (Item 8 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13432900 EMBASE No: 2005495191
Fourth round table conference in Monaco on 15 January 2005: Regulation of muscle growth, a therapeutic issue for Duchenne muscular dystrophy?
Scheuerbrandt G.
Dr. G. Scheuerbrandt, Im Talgrund 2, D-769874 Breitnau Germany
AUTHOR EMAIL: gscheuerbrandt@t-online.de
Acta Myologica (ACTA MYOLOGICA) (Italy) 2005, 24/1 (25-35)
CODEN: ACMYF ISSN: 1128-2460
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: ENGLISH

2/7/52 (Item 9 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13319975 EMBASE No: 2005400964
The therapeutic potential of agents that inactivate %myostatin%
Bishop A.; Kambadur R.; Sharma M.
M. Sharma, AgResearch, East Street, Private Bag 3123, Hamilton New Zealand
AUTHOR EMAIL: Mridula.Sharma@agresearch.co.nz
Expert Opinion on Investigational Drugs (EXPERT OPIN. INVEST. DRUGS) (United Kingdom) 2005, 14/9 (1099-1106)
CODEN: EOIDE ISSN: 1354-3784
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 67

%Myostatin% is a member of the TGF-beta superfamily of secreted growth factors. A lack of functional %myostatin% or inhibition of the normal %myostatin% function results in an increased muscling phenotype and, conversely, the systemic administration of %myostatin% results in muscle wasting. Thus, %myostatin% is well established as a negative regulator of skeletal muscle mass. %Myostatin% binds to cell-surface receptors to inhibit both the proliferation and differentiation of myoblasts. Moreover, it functions to regulate both embryonic and post-natal musculature. Thus, potential antagonists to %myostatin%, whether targeting %myostatin% synthesis, secretion or receptor binding, show great promise as therapies against muscle-wasting diseases. This review provides an expert opinion on the biology and potential of %myostatin% antagonists in the treatment of muscle-wasting disorders. (c) 2005 Ashley Publications Ltd.

2/7/53 (Item 10 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13153795 EMBASE No: 2005217897
%Myostatin%, a negative regulator of muscle mass: Implications for muscle degenerative diseases
Tobin J.F.; Celeste A.J.
J.F. Tobin, Department of Cardiovascular and Metabolic Diseases, Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140 United States
AUTHOR EMAIL: jtobin@wyeth.com
Current Opinion in Pharmacology (CURR. OPIN. PHARMACOL.) (United Kingdom) 2005, 5/3 SPEC. ISS. (328-332)
CODEN: COPUB ISSN: 1471-4892
PUBLISHER ITEM IDENTIFIER: S1471489205000366
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 41

%Myostatin% is a secreted protein that negatively regulates skeletal

muscle mass determining both muscle fiber number and size. The %myostatin% pathway is conserved and regulates muscle mass in a number of animal species ranging from fish to humans. Inhibition of %myostatin% using a variety of therapeutic approaches can increase muscle mass in a number of animal models of human disease, including muscular dystrophy. (c) 2005 Elsevier Ltd. All rights reserved.

2/7/54 (Item 11 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

13006923 EMBASE No: 2005066656
The function of %Myostatin% and strategies of %Myostatin% blockade - New hope for therapies aimed at promoting growth of skeletal muscle
Patel K.; Amthor H.
K. Patel, Dept. of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 OTU United Kingdom
AUTHOR EMAIL: kpatel@rvc.ac.uk
Neuromuscular Disorders (NEUROMUSCULAR DISORD.) (United Kingdom) 2005 , 15/2 (117-126)
CODEN: NEDIE ISSN: 0960-8966
PUBLISHER ITEM IDENTIFIER: S0960896604003098
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 73

Genetic deletion of %Myostatin%, a member of the Transforming Growth Factor-beta family of signalling molecules, resulted in excessive growth of skeletal muscle. It demonstrated the remarkable intrinsic growth potential of skeletal muscle and led to the proposal that growth stimulation could amend diseased muscle without having to correct the primary cause of the disease. Furthermore, the presence of %Myostatin% in skeletal muscle in a number of muscle diseases and disease models suggested that it aggravated the primary pathology. Inhibition of %Myostatin% activity in mdx mouse, the animal model for Duchenne muscular dystrophy, resulted in increased force production and better tissue architecture which implicated %Myostatin% as a target for new therapeutic strategies. In this review we will discuss the phenotypes of animal models in which %Myostatin% function is altered. We will highlight the particularities of the %Myostatin% signalling pathway and describe molecular strategies that have been developed to inhibit the function of %Myostatin% on muscle. Finally, we will summarise the role of %Myostatin% in diseased muscle and discuss blockade of %Myostatin% as a potential therapy for muscular dystrophies. (c) 2004 Elsevier B.V. All rights reserved.

2/7/55 (Item 12 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12956493 EMBASE No: 2005016887
Between the covers: This month's main articles
Aminoff M.J.
Dr. M.J. Aminoff, Department of Neurology, Univ. of California - San Francisco, San Francisco, CA United States
Muscle and Nerve (MUSCLE NERVE) (United States) 2005, 31/1 (v-vi)
CODEN: MUNED ISSN: 0148-639X
DOCUMENT TYPE: Journal ; Editorial
LANGUAGE: ENGLISH

2/7/56 (Item 13 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12939552 EMBASE No: 2004529758
Emerging drugs for sarcopenia: Age-related muscle wasting
Lynch G.S.
Dr. G.S. Lynch, Department of Physiology, Centre for Neuroscience, University of Melbourne, Melbourne, Vic. 3010 Australia
AUTHOR EMAIL: gsl@unimelb.edu.au
Expert Opinion on Emerging Drugs (EXPERT OPIN. EMERG. DRUGS) (United Kingdom) 2004, 9/2 (345-361)
CODEN: EOEDA ISSN: 1472-8214
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 184

Sarcopenia is the term widely used to describe the progressive loss of muscle mass with advancing age. Even before significant muscle wasting becomes apparent, ageing is associated with a slowing of movement and a gradual decline in muscle strength, factors that increase the risk of injury from sudden falls and the reliance of the frail elderly on assistance in accomplishing even basic tasks of independent living. Sarcopenia is recognised as one of the major public health problems now facing industrialised nations, and its effects are expected to place increasing demands on public healthcare systems worldwide. Although the effects of ageing on skeletal muscle are unlikely to be halted or reversed, the underlying mechanisms responsible for these deleterious changes present numerous targets for drug discovery with potential opportunities to attenuate muscle wasting, improve muscle function, and preserve functional independence. Very few drugs have been developed with Sarcopenia specifically in mind. However, because many of the effects of ageing on skeletal muscle resemble those indicated in many neuromuscular disorders, drugs that target neurodegenerative diseases may also have important relevance for treating age-related muscle wasting and weakness. This review describes a selection of the emerging drugs that have been developed during the period 1997-2004, relevant to Sarcopenia.

2/7/57 (Item 14 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12834375 EMBASE No: 2004432295
Metabolic Diseases Drug Discovery - Strategic Research Institute's Third International World Summit. Dipeptidyl peptidase-IV inhibitors
Xu J.
J. Xu, BioMinerva Group, 11553 Hadar Drive, San Diego, CA 92126 United States
AUTHOR EMAIL: jxu4@san.rr.com
IDrugs (IDRUGS) (United Kingdom) 2004, 7/9 (839-840)
CODEN: IDRUF ISSN: 1369-7056
DOCUMENT TYPE: Journal ; Conference Paper
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

The majority of the presentations at the conference were on three highly sought-after targets for type 2 diabetes mellitus, namely PTP1B, PPARs and DPP-IV, reflecting the current focus and trend in the industry. A couple of novel targets were discussed, including the potential of %myostatin% as a type 2 diabetes mellitus target and a novel GPCR target. While small molecules were dominant, several biological based approaches were covered: %antibody% therapeutics and oligonucleotide-based approaches (ASO and siRNA). In searching for small-molecule leads, structure-based rational design and focused combination chemistry appear to produce better results than a random high-throughput approach over the entire chemical library. The biggest challenges for diabetes and obesity drugs remain similar to those mentioned in previous meetings: increasing specificity to reduce side effects and maintaining long-term effect while maintaining or increasing efficacy. Due to the tremendous interest of the pharmaceutical industry in metabolic disease drug development, our knowledge of food intake and metabolism regulation has increased exponentially. Overall, the prospect of better drugs for, and better control of, type 2 diabetes mellitus and obesity is promising. (c) The Thomson Corporation.

2/7/58 (Item 15 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12824796 EMBASE No: 2004420594
New therapies for muscular dystrophy: Cautious optimism
Cossu G.; Sampaolesi M.
AUTHOR EMAIL: cossu.giulio@hsr.it
Trends in Molecular Medicine (TRENDS MOL. MED.) (United Kingdom) 2004, 10/10 (516-520)
CODEN: TMMRC ISSN: 1471-4914
PUBLISHER ITEM IDENTIFIER: S1471491404002151
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 47

The quest for a therapy for muscular dystrophy has been the driving force behind the past 40 years of advances in this field. Numerous results, such as the identification of satellite cells and gene mutations that are responsible for most forms of dystrophies, advances in gene transfer and modification technology and, more recently, stem cells, have fueled hopes. However, administering corticosteroids still remains the only effective treatment available. Several recent advances have uncovered a diversity of possible therapeutic approaches, from pharmacological treatments to gene therapy (exon-skipping and adeno-associated viruses) and cell therapy with different types of newly identified stem cells. Importantly, a combination of these strategies might greatly enhance the possibility of successful therapy.

2/7/59 (Item 16 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12806585 EMBASE No: 2004400291
Update on therapies for sarcopenia: Novel approaches for age-related muscle wasting and weakness
Lynch G.S.
Prof. G.S. Lynch, Department of Physiology, The University of Melbourne, Centre for Neuroscience, Melbourne, Vic. 3010 Australia
AUTHOR EMAIL: gsl@unimelb.edu.au
Expert Opinion on Therapeutic Patents (EXPERT OPIN. THER. PAT.) (United Kingdom) 2004, 14/9 (1329-1344)
CODEN: EOTPE ISSN: 1354-3776
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 156

Sarcopenia is the term widely used to describe the slow, but progressive, loss of muscle mass with advancing age. Even before significant muscle wasting becomes apparent, ageing is associated with a slowing of movement and a gradual decline in muscle strength, factors that increase the risk of injury from sudden falls, and a reliance on the frail elderly for assistance in accomplishing even basic tasks of independent living. Sarcopenia affects everyone regardless of their ethnicity, gender or wealth, and it is recognised as one of the major public health problems now facing industrialised nations. Although many argue that the effects of ageing on skeletal muscle are immutable and irreversible, such a view does not take into consideration the range of different approaches currently under investigation that could, theoretically, and perhaps realistically, improve muscle function and preserve functional independence. This review provides an update on progress regarding the development of therapies for sarcopenia. It expands on the information provided in an earlier review and covers the most recent (2002 - 2004) patents on novel approaches relevant to the treatment of age-related muscle wasting and weakness. Although there have been many therapies proposed for sarcopenia, and many patents awarded for such claims, unfortunately very few have data to support their assertions, and even fewer have sufficient data to warrant potential clinical application. 2004 (c) Ashley Publications Ltd.

2/7/60 (Item 17 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12795515 EMBASE No: 2004389494
Pneumatic resistance machines can provide eccentric loading (multiple letters)
Roth S.M.; Rogers M.A.; Hurley B.F.; Martel G.F.; Willoughby D.
S.M. Roth, Department of Kinesiology, Coll. of Hlth. and Human Performance, University of Maryland, College Park, MD United States
Medicine and Science in Sports and Exercise (MED. SCI. SPORTS EXERC.) (United States) 2004, 36/9 (1655-1656)
CODEN: MSCSB ISSN: 0195-9131
DOCUMENT TYPE: Journal ; Letter
LANGUAGE: ENGLISH

2/7/61 (Item 18 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12666616 EMBASE No: 2004262124
%Myostatin% mutation associated with gross muscle hypertrophy in a child
Schuelke M.; Wagner K.R.; Stolz L.E.; Hubner C.; Riebel T.; Komen W.; Braun T.; Tobin J.F.; Lee S.-J.
Dr. M. Schuelke, Department of Neuro-Pediatrics, Charite, University Medical Center Berlin, Augustenburger Platz 1, D-13353 Berlin Germany
AUTHOR EMAIL: markus.schuelke@charite.de
New England Journal of Medicine (NEW ENGL. J. MED.) (United States) 24 JUN 2004, 350/26 (2682-2688)
CODEN: NEJMA ISSN: 0028-4793
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 23

2/7/62 (Item 19 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12528255 EMBASE No: 2004112032
Therapeutics for Duchenne muscular dystrophy: Current approaches and future directions
Bogdanovich S.; Perkins K.J.; Krag T.O.B.; Khurana T.S.
T.S. Khurana, Department of Physiology, Pennsylvania Muscle Institute, Univ. of PA School of Medicine, 3700 Hamilton Walk, Richards A-601, Philadelphia, PA 19104-6085 United States
AUTHOR EMAIL: tsk@mail.med.upenn.edu
Journal of Molecular Medicine (J. MOL. MED.) (Germany) 2004, 82/2 (102-115)
CODEN: JMLME ISSN: 0946-2716
DOCUMENT TYPE: Journal ; Review
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 177

Duchenne muscular dystrophy (DMD) is the most common X-linked neuromuscular disorder. The devastating nature of DMD has led to an intense effort toward finding a cure for this disease, dating back to the time when Duchenne first initiated clinical trials using faradic stimulation for DMD patients. Unfortunately despite the passage of some 150 years the disease remains incurable, and its medical management is largely supportive. However, the discovery of the DMD gene about 20 years ago has allowed a change in the focus of therapeutic strategy dramatically toward delivery of the missing gene/protein. Indeed, some degree of success has been achieved in preclinical animal studies using such strategies, and gene therapy trials are currently underway in humans. Pharmacological approaches for DMD are also being developed since they can circumvent some of the technical problems associated with gene and cell based therapy. This review explores developments in therapeutic approaches for DMD.

2/7/63 (Item 20 from file: 73)

DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12475222 EMBASE No: 2004063739
%Myostatin% inhibits rhabdomyosarcoma cell proliferation through an Rb-independent pathway
Langley B.; Thomas M.; McFarlane C.; Gilmour S.; Sharma M.; Kambadur R.
R. Kambadur, Animal Genomics, AgResearch, East Street, Hamilton New Zealand
AUTHOR EMAIL: Ravi.Kambadur@agresearch.co.nz
Oncogene (ONCOGENE) (United Kingdom) 15 JAN 2004, 23/2 (524-534)
CODEN: ONCNE ISSN: 0950-9232
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 61

Rhabdomyosarcoma (RMS) tumors are the most common soft-tissue sarcomas in childhood. In this investigation, we show that %myostatin%, a skeletal muscle-specific inhibitor of growth and differentiation is expressed and translated in the cultured RMS cell line, RD. The addition of exogenous recombinant %myostatin% inhibits the proliferation of RD cells cultured in growth media, consistent with the role of %myostatin% in normal myoblast proliferation inhibition. However, unlike normal myoblasts, upregulation of p21 was not observed. Rather, %myostatin% signalling resulted in the specific downregulation of both Cdk2 and its cognate partner, cyclin-E. The analysis of Rb reveals that there was no change in its phosphorylation status with %myostatin% treatment, consistent with D-type-cyclin-Cdk4/6 complexes being active in the absence of p21. Moreover, the activity of Rb appeared to be unchanged between treated and nontreated RD cells, as determined by the ability of Rb to bind E2F1. The examination of NPAT, a substrate of cyclin-E-Cdk2 involved in the transcriptional activation of replication-dependent histone gene expression, revealed that it undergoes a loss of phosphorylation with %myostatin% treatment. Supporting this, a downregulation in H4-histone gene expression was observed. These results suggest that %myostatin% could potentially be used as an inhibitor of RMS proliferation and define a previously uncharacterized, Rb-independent mechanism for the inhibition of muscle precursor cell proliferation by %myostatin%.

2/7/64 (Item 21 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

12331904 EMBASE No: 2003447971
Body building best without %myostatin%
Brazil M.
Nature Reviews Drug Discovery (NAT. REV. DRUG DISCOV.) (United Kingdom) 2003, 2/1 (6)
CODEN: NRDDA ISSN: 1474-1776
DOCUMENT TYPE: Journal ; Note
LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 1

2/7/65 (Item 22 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

11904538 EMBASE No: 2003007233
Sizing up muscular dystrophy
Zammit P.S.; Partridge T.A.
P.S. Zammit, Faculty of Medicine, Imperial College, Hammersmith Hospital Campus, London United Kingdom
AUTHOR EMAIL: terence.partridge@csc.mrc.ac.uk
Nature Medicine (NAT. MED.) (United States) 01 DEC 2002, 8/12 (1355-1356)
CODEN: NAMEF ISSN: 1078-8956
DOCUMENT TYPE: Journal ; Short Survey
LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 12

2/7/66 (Item 23 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2006 Elsevier B.V. All rts. reserv.

11878571 EMBASE No: 2002450398
New muscular dystrophy treatment tested in mice
Bradbury J.
Lancet (LANCET) (United Kingdom) 30 NOV 2002, 360/9347 (1756)
CODEN: LANCA ISSN: 0140-6736
DOCUMENT TYPE: Journal ; Short Survey
LANGUAGE: ENGLISH

2/7/67 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2006 The Thomson Corp. All rts. reserv.

0412581 DBR Accession No.: 2006-26077 PATENT
New Immunoglobulin G Fc fragment useful as drug carrier, which is modified by non-peptide polymer - involving vector-mediated gene transfer and expression in host cell for use in drug screening
AUTHOR: KIM Y; BAE S; KIM D; SONG D; LIM C; KWON S; LEE G
PATENT ASSIGNEE: HANMI PHARM CO LTD 2006
PATENT NUMBER: WO 2006107124 PATENT DATE: 20061012 WPI ACCESSION NO.: 2006-717605 (200674)
PRIORITY APPLIC. NO.: KR 29666 APPLIC. DATE: 20050408
NATIONAL APPLIC. NO.: WO 2005KR1233 APPLIC. DATE: 20050428
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An immunoglobulin G (IgG) Fc fragment as a drug carrier, which is modified by a non-peptide polymer is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a pharmaceutical composition comprising an Fc fragment modified by a non-peptide polymer as carrier; (2) a complex in which an Fc fragment modified by a non-peptide polymer is linked to a drug via a linker; and (3) a pharmaceutical composition for increasing in vivo duration of action an in vivo stability of a drug, comprising the complex above and an acceptable carrier. BIOTECHNOLOGY - Preferred Component: The physiologically active polypeptide comprises hormones, cytokines, enzymes, %antibodies%, growth factors, transcription regulatory factors, coagulation factors, vaccines, structural proteins, ligand proteins, receptors, cell surface antigens, or receptor antagonists. It may also comprise human growth hormone, growth hormone releasing hormone, growth hormone releasing peptide, interferons, interferon receptors, colony stimulating factors, glucagon-like peptides (e.g., GLP-1), C-protein-coupled receptor, interleukin binding proteins, cytokine binding proteins, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, allergy inhibitor, cell necrosis glycoproteins, immunotoxin, lymphotoxin, tumor necrosis factor, tumor suppressors, metastasis growth factor, alpha-1, antitrypsin, albumin, alpha-lactalbumin, apolipoprotein-E, erythropoietin, highly glycosylated erythropoietin, angiopoietins, hemoglobin, thrombin, thrombin receptor activating peptide, thrombomodulin, factor VII, factor VIIa, factor VIII, factor IX, factor XIII, plasminogen activating factor, fibrin-binding peptide, urokinase, streptokinase, hirudin, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, leptin, platelet-derived growth factor, epithelial growth factor, epidermal growth factor, angiostatin, angiotensin, bone growth factor, bone stimulating protein, calcitonin, insulin, atriopeptin, cartilage inducing factor, elcatonin, connective tissue activating factor, tissue factor pathway inhibitor, follicle stimulating hormone, luteinizing hormone, luteinizing hormone releasing hormone, nerve growth factors, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocortical hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, autotaxin, lactoferrin, %myostatin%, receptors, receptor antagonists, cell surface antigens, monoclonal %antibodies%, polyclonal

%antibodies%, or %antibody% fragments (preferably human growth hormone, colony stimulating factors, interferon-alpha, or erythropoietin). The IgG is IgG2 or IgG4. The Fc fragment is an aglycisylated IgG4 Fc fragment. Preferred Source: The Fc fragment is derived from IgG, IgA, IgD, IgE, and/or IgM, or their hybrids; or derived from IgG1, IgG2, IgG3, and/or IgG4, or their hybrids. USE - Useful as a drug carrier. ADVANTAGE - The inventive Fc fragment modified by a non-peptide lacks immunogenicity and effector functions, allowing Fc fragment to maintain in vivo activity of a drug conjugated in high levels, remarkably increasing the serum half-life of the drug, and remarkably reducing the risk of inducing immune responses. EXAMPLE - Immunoglobulin G (IgG) (150-kDa, 200 mg) dissolved in 10 mM phosphate buffer was treated with a proteolytic enzyme, papain (2 mg) at 37degreesC for 2 hours with gentle agitation regenerating the native immunoglobulin Fc fragment. The regenerated Fc fragment was subjected to chromatography for purification. Human immunoglobulin IgG4 heavy chain constant regions, a derivative (dCysG4Fc), having a nine amino acid deletion at the amino terminum of the native hinge region was prepared. An expression vector containing Escherichia coli secretory sequence, pT14S1SH-4T20V22Q was used. To obtain human immunoglobulin IgG4 heavy chain constant regions, RT-PCR was carried out using RNA isolated from human blood cells as a template. To clone each of the amplified IgG4 constant region fragments into an expression vector containing E. coli secretory sequence variant, pT14S1SH-4T20V22Q was used. The constructed expression vector was transformed into an expression host cell, E. coli BL21(DE3). An inducer, IPTG, was added to culture to induce protein expression. E. coli cells recovered from the fermentation fluid were disrupted to provide cell lysates. The cell lysates were subjected to 2-step column chromatography to purify recombinant immunoglobulin constant region derivatives present in the cytosol. Polyethylene glycol succinimidyl propionate (PEG-SPA) and polyethylene glycol N-hydroxysuccinimidyl were individually mixed with 100 mg of recombinant (dCysG4Fc) carrier in 20 of tris-hydrochloric acid buffer at a carrier:PEG molar ratio of 1:2. The reaction mixture was allowed to react at 4degreesC for 2 hours, and mono-pegylated carriers and di-pegylated carriers were purified.(83 pages)

2/7/68 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0410919 DBR Accession No.: 2006-24415 PATENT
New chimeric insulin-like growth factor polypeptide, useful for treating a disease, e.g. muscle atrophy, dwarfism, or myocardial infarction - for use in insufficiency disease, insulin-like growth factor disorder, muscle atrophy, dwarfism, myocardial infarction, aging, AIDS-induced cachexia, vurnerary, cancer-induced cachexia, casting, congestive heart failure, diabetes, growth hormone deficiency, inflammation, rheumatoid arthritis, kidney failure, sarcopenia and sepsis-induced cachexia therapy and gene therapy
AUTHOR: GLASS D J
PATENT ASSIGNEE: GLASS D J 2006
PATENT NUMBER: US 20060223753 PATENT DATE: 20061005 WPI ACCESSION NO.: 2006-668904 (200669)
PRIORITY APPLIC. NO.: US 395706 APPLIC. DATE: 20060331
NATIONAL APPLIC. NO.: US 395706 APPLIC. DATE: 20060331
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A chimeric insulin-like growth factor (IGF) polypeptide, comprising an IGF2 derived component comprising amino acid 7-37 or 3-64 of IGF2 (sequence comprising 67 amino acids, SEQ ID NO: 3) and an IGF1 derived component comprising amino acid 38-64 or 4-36 of IGF1 (sequence comprising 70 amino acids, SEQ ID NO: 1), and optionally a fusion component (F) and/or a signal sequence, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: (1) a dimer of the chimeric polypeptide above; (2) a nucleic acid which encodes a chimeric polypeptide above; (3) a vector comprising the nucleic acid; (4) a method of producing the chimeric polypeptide above; (5) a pharmaceutical composition comprising the chimeric polypeptide above and a pharmaceutical carrier; and (6) a therapeutic method for the treatment of an IGF1 insufficiency disease or condition, or a condition which is ameliorated or improved with IGFI. BIOTECHNOLOGY - Preferred Chimeric Polypeptide: The F is a multimerizing component, where the multimerizing component is an immunoglobulin domain, and where the multimerizing component is the Fc domain of human IgG. The F is a second active or therapeutic agent. The second active or therapeutic agent is an activating %antibody% or a blocking %antibody%, where the blocking %antibody% blocks a component selected from %myostatin%, activin receptor, BMP receptor I, TNF receptor, IL-1 receptor, ALK3 receptor, or ALK4 receptor and the activating %antibody% activates a component selected from IGF1 receptor, B2 adrenergic receptor, or the IL-15 receptor complex. The second active or therapeutic agent is a ligand for a cell surface receptor, and is capable of binding and activating the cell surface receptor, where the active or therapeutic agent is IL-15, myotrophin, urocortin, urocortin II, insulin, human %myostatin% propeptide, hGH, proliferin, follistatin, FSTL1, and FLRG, or its mutant, derivative, or fragment having biological activity. The F is a muscle targeting ligand, e.g. agrin or its variants or mutants that bind MusK, muscle cadherin, or its N-terminal domains that bind homophilic muscle cadherins. A chimeric IGF polypeptide comprises a polypeptide having a sequence comprising 58, 309, 59, or 334 amino acids (ODD SEQ ID NOS: 5-11). Preparation (claimed): Producing the chimeric polypeptide above comprises culturing a host cell transfected with a vector comprising a nucleic acid molecule of the invention, under conditions for expression of the protein from the host cell, and recovering the polypeptide so produced. Preferred Method: Treating an IGF1 insufficiency disease or condition, or a condition, which is ameliorated or improved with IGFI, comprises administering a therapeutic amount of the pharmaceutical composition of (5) to a subject, or a subject at risk for development of that disease or condition. The IGFI disease or condition is muscle atrophy, dwarfism, or myocardial infarction, where muscle atrophy is a result of aging, AIDS-induced cachexia, burns, cancer-induced cachexia, casting, congestive heart failure, settings where inflammatory cytokines such as IL-1 or TNF-alpha are in excess, denervation, diabetes, disuse (such as in prolonged bed rest), growth hormone deficiency, IGF1 deficiency, immobilization, inflammation, such as in chronic inflammatory conditions such as rheumatoid arthritis, mechanic ventilation (resulting in atrophy of the diaphragm), renal failure, sarcopenia, and sepsis-induced cachexia. ACTIVITY - Muscular-Gen; Endocrine-Gen; Cardiant. MECHANISM OF ACTION - Gene Therapy. USE - The polypeptide, composition, and method are useful for treating IGFI disease or condition, e.g. muscle atrophy, dwarfism, or myocardial infarction. ADMINISTRATION - Administration is by enteral or parenteral and including intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, pulmonary, intranasal, intraocular, epidural, or oral routes. No dosage details given. EXAMPLE - No suitable example given.(14 pages)

2/7/69 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0406620 DBR Accession No.: 2006-20116 PATENT
Improving tissue wound healing by administering one or more %myostatin% antagonist to human or non-human patient - involving recombinant vector-mediated %myostatin% gene transfer and expression in host cell for use in tissue vulnerary and fibrotic disease gene therapy
AUTHOR: KAMBADUR R; SHARMA M; HENNEBRY A; SENNA SALERNO DE MOURA M
PATENT ASSIGNEE: OVITA LTD 2006
PATENT NUMBER: WO 200683182 PATENT DATE: 20060810 WPI ACCESSION NO.: 2006-550291 (200656)
PRIORITY APPLIC. NO.: NZ 205Z-538097 APPLIC. DATE: 20050207
NATIONAL APPLIC. NO.: WO 2006NZ9 APPLIC. DATE: 20060207
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Improving (M1) tissue wound healing, by administering at least one %myostatin% antagonist to a human or non-human patient. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a pharmaceutical compound comprising at least one

%myostatin% antagonist and a carrier; and (2) at least one %myostatin% antagonist for improving tissue wound healing in a human or non-human patient. BIOTECHNOLOGY - Preferred Method: In the method (M1), at least one %myostatin% antagonist is chosen from an anti-%myostatin% %antibody%, a %myostatin% peptide immunogen, %myostatin% multimer or %myostatin% immuno-conjugate capable of eliciting an immune response and blocking %myostatin% activity, a protein inhibitor of %myostatin% chosen from a truncated Activin type II receptor, a %myostatin% pro-domain and follistatin, or a functional fragment of the protein inhibitor, a %myostatin% inhibitor released into culture from cells overexpressing %myostatin%, a dominant negative of %myostatin% selected from the Piedmontese allele and mature %myostatin% peptides having a C-terminal truncation at a position at or between amino acid positions 335-375, a small peptide comprising the amino acid sequence Trp-Met-Cys-Pro-Pro and which is capable of binding to and inhibiting %myostatin%, a splice variant of %myostatin%, a regulator of the %myostatin% pathway, and an antisense polynucleotide, RNAi, siRNA or an anti-%myostatin% ribozyme capable of inhibiting %myostatin% activity by inhibiting %myostatin% gene expression, preferably at least one %myostatin% antagonist is a dominant negative of %myostatin% chosen from the Piedmontese allele and mature %myostatin% peptides having a C-terminal truncation at or between amino acid positions 335-375, more preferably mature %myostatin% peptide having a C-terminal truncation at amino acid position 335 or 350. The %myostatin% antagonist is a splice variant of %myostatin% chosen from a polypeptide having a fully defined 321 amino acid (SEQ ID No. 8), 65 amino acid (SEQ ID No. 9), 47 amino acid (SEQ ID No. 10), 321 amino acid (SEQ ID No. 11), 65 amino acid (SEQ ID No. 12), 47 amino acid (SEQ ID No. 13) or 314 amino acid (SEQ ID No. 14) sequence given in the specification, or its functional fragment or variant, or a sequence having 95%, 90%, 85%, 80%, 75% or 70% sequence identity to it. The %myostatin% antagonist is a regulator of the %myostatin% pathway comprising the mighty peptide having any one of the 2 fully defined 192 amino acid (SEQ ID No. 16 or 18) sequences given in the specification, or its functional fragment or variant, or a sequence having 95%, 90%, 85%, 80%, 75% or 70% sequence identity to it. The additional immuno-responsive compounds chosen from glucocorticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), platelet derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and tumor necrosis factor (TNF)-alpha antagonists are co-administered either separately, sequentially or simultaneously with the %myostatin% antagonist to further improve wound healing. ACTIVITY - Vulnerary; Antiinflammatory. MECHANISM OF ACTION - %Myostatin%-Antagonist; Gene Therapy. One year old wild type mice (C57 black) were injured with notexin and administered with %myostatin% antagonists such as 350 or with saline (control). After a notexin type wounding, the muscle weight initially increases due to the resulting edema, followed by a decrease due to necrosis of the damaged muscle fibers that are cleared from the site of wounding. The muscle weight was monitored. The result showed that %myostatin% antagonist 350 treated mice do not lose as much weight as mice treated with control saline. USE - The method (M1) and the pharmaceutical compound are useful for improving tissue wound healing, preferably superficial skin wound including cuts and abrasions, deep wound extending through the skin and muscle including surgical incisions, internal wounds including wounds to muscle and tendon caused by sports injury or trauma, bruises and hematomas and burns. The %myostatin% antagonist is useful in the manufacture of a medicament for improving tissue wound healing in a human or non-human patient and for treating fibrotic diseases or disorders (all claimed). ADMINISTRATION - The %myostatin% antagonist is administered by local or systemic, preferably oral, intravenous, cutaneous, subcutaneous, intradermal, topical, nasal, pulmonary, intramuscular or intraperitoneal route (claimed). No dosage details given. ADVANTAGE - The method (M1) significantly improves tissue wound healing, and reduces scar tissue formation or loss of tissue function.(75 pages)

2/7/70 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0405112 DBR Accession No.: 2006-18608 PATENT
New fusion protein comprising GDF8 (growth and differentiation factor 8) peptide domain and plant virus coat protein, useful for eliciting an anti-GDF8 immune response - involving vector-mediated gene transfer and expression in tobacco transgenic plant construction
AUTHOR: SMITH M L; PALMER K E; POGUE G P; JUNKER D L; COCHRAN M D
PATENT ASSIGNEE: SCHERING-PLOUGH LTD 2006
PATENT NUMBER: WO 200673827 PATENT DATE: 20060713 WPI ACCESSION NO.: 2006-522201 (200653)
PRIORITY APPLIC. NO.: US 665690 APPLIC. DATE: 20050328
NATIONAL APPLIC. NO.: WO 2005US46363 APPLIC. DATE: 20051221
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A fusion protein comprising a GDF8 (also known as %myostatin%) peptide domain, or an antigenic fragment of the GDF8 peptide domain, where the GDF8 peptide domain comprises amino acid residues 327-346 of a sequence comprising 375 amino acids (SEQ ID NO: 1), and a plant virus coat protein, or at least one fragment of a plant virus coat protein, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a nucleic acid that encodes the fusion protein above; (2) a replicable vector comprising the nucleic acid; (3) a host cell comprising the replicable vector; (4) a tobacco plant that comprises the host cell; (5) a method of producing a fusion protein comprising a GDF8 peptide domain or an antigenic fragment of a GDF8 peptide domain; (6) a vaccine composition comprising the fusion protein above; (7) a method of eliciting an anti-GDF8 immune response in an animal; and (8) a method of down-regulating GDF8 activity in an animal. BIOTECHNOLOGY - Preferred Sequences: The plant virus coat protein is a tobacco virus coat protein, where the GDF8 peptide domain or the antigenic fragment of the GDF8 peptide domain is fused to a fragment of the coat protein, and where the tobacco virus is a tobacco mosaic virus strain U1 or U5. The fusion protein comprises an amino acid sequence of SEQ ID NOS: 47-51, 54, or 55. The antigenic fragment of the GDF8 peptide domain comprises amino acid residues 329-332, preferably amino acid residues 327-338, of SEQ ID NO: 1. The coat protein is from a single-stranded plus-sense plant RNA virus. The GDF8 peptide domain is fused to the coat protein at a position selected from the N-terminus of the coat protein, the C-terminus of the coat protein, four amino acids from the C-terminus of the coat protein, and within an externally exposed loop region of the coat protein, where the fusion protein elicits an immune response to GDF8, with or without an adjuvant. The X3DF8 peptide domain comprises one or more amino acid substitutions, where there are no more than five amino acid substitutions between amino acid residues 327-346 of SEQ ID NO: 1, and where the fusion protein specifically binds to rat monoclonal %antibody% 788. The GDF8 peptide domain comprises amino acid substitutions at a position selected from residues 328, 329, 331, 333, 335 of SEQ ID NO: 1, or their combination(s), where, amino acid residue 328 is His, Leu, or Asn; amino acid residue 329 is Gln or Lys; amino acid residue 331 is Asn or Ser; amino acid residue 333 is Arg or Lys; or amino acid residue 335 is Ser, Pro, or Thr. The fusion protein comprises no more than one amino acid substitution between residues 327-346, provided that the fusion protein specifically binds to rat monoclonal %antibody% 788. The fusion protein comprises a specific neutralization epitope for an anti-GDF8 %antibody%, where the anti-GDF8 %antibody% is rat anti-GDF8 monoclonal %antibody% 788 or an IgG fraction of goat anti-GDF8 polyclonal antiserum. The fusion protein elicits an immune response to GDF8 when presented to the immune system of a vertebrate, with or without an adjuvant. The fusion protein comprises an antigenic fragment of a GDF8 peptide that comprises 4-16 consecutive amino acid residues from human GDF8. The nucleic acid comprises nucleotide 1112-1171 of a sequence comprising 2823 bp (SEQ ID NO: 2). Preferred Replicable Vector: The replicable vector is a plasmid, a phage, a cosmid, or a virus. The replicable vector is a tobacco virus selected from TMV-FV1, TMV-FV2, TMV-FV3, TMV-FV4, TMV-FV5, TMV-FV6 or TMV-FV7. Preferred Host Cell: The host cell is a plant cell. Preferred Methods: Producing a fusion protein comprising a GDF8 peptide domain or an antigenic fragment of a GDF8 peptide domain comprises culturing the host cell of (4), and expressing the encoded fusion protein. The method further comprises recovering the fusion protein. Producing a fusion

protein comprising a GDF8 peptide domain or an antigenic fragment of a GDF8 peptide domain comprises infecting a tobacco plant with the tobacco mosaic virus, where the tobacco mosaic virus replicates, and harvesting the replicated tobacco mosaic virus. The method further comprises isolating the fusion protein from the harvested plant virus. Eliciting an anti-GDF8 immune response in an animal comprises administering to the animal an amount of the vaccine composition of (6). Down-regulating GDF8 activity in an animal comprises immunizing the animal with an amount of the vaccine composition of (6). Preferred Vaccine Composition: The vaccine composition further comprises an adjuvant. ACTIVITY - Immunostimulant; Antidiabetic; Anabolic. No biological data given. MECHANISM OF ACTION - Vaccine. USE - The fusion protein, vaccine composition, and method are useful for eliciting an anti-GDF8 immune response, useful in enhancing growth of muscle mass in food animals and in treating Type II diabetes. ADMINISTRATION - Dosage 1 mg/kg - 1 g/kg by parenteral means. EXAMPLE - No relevant example given.(139 pages)

2/7/71 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2006 The Thomson Corp. All rts. reserv.

0397471 DBR Accession No.: 2006-10967 PATENT
New composition of matter, useful for modifying Fc domain that is already linked through an N- or C-terminus or side chain to a peptide or to a polypeptide - composition of matter and vector expression in host cell for use in Fc domain modification
AUTHOR: GEGG C; XIONG F; SITNEY K C
PATENT ASSIGNEE: AMGEN INC 2006
PATENT NUMBER: WO 200636834 PATENT DATE: 20060406 WPI ACCESSION NO.: 2006-263854 (200627)
PRIORITY APPLIC. NO.: US 612680 APPLIC. DATE: 20040924
NATIONAL APPLIC. NO.: WO 2005US34273 APPLIC. DATE: 20050923
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A composition of matter comprising formula (I), or its multimers is new. DETAILED DESCRIPTION - The composition of matter comprises the formula (X1)a-F1-(X2)b (I), F1 = an Fc domain modified so that it comprises at least one X3 in a loop region; X1 and X2 = each independently selected from linkers (L1-L8) combined with P1-P8, given in the specification; P1, P2, P3, and P4 = each independently sequences of pharmacologically active polypeptides or pharmacologically active peptides; P5, P6, P7, and P8 are each independently sequences of pharmacologically active peptides; and L1, L2, L3, L4, L5, L6, L7, and L8 are each independently linkers; and a, b, c, d, e, and f = each independently 0 or 1. INDEPENDENT CLAIMS are also included for: (1) a DNA encoding the composition of matter; (2) an expression vector comprising the DNA; (3) a host cell comprising the expression vector; (4) a process for preparing a pharmacologically active compound, by selecting at least one randomized peptide that modulates the activity of a protein of interest; and preparing a pharmacologic agent comprising an amino acid sequence of the selected peptide as an internal sequence of an Fc domain; (5) a modified %antibody% , comprising an Fc domain modified so that it comprises at least one X3 in a loop region; and (6) a process for preparing a modified %antibody% , which comprises: selecting at least one peptide that modulates the activity of a protein of interest; and preparing an %antibody% comprising an amino acid sequence of the selected peptide in a loop region of an Fc domain of the %antibody%. BIOTECHNOLOGY - Preferred Composition: The composition of matter comprises the Fc domain comprising an IgG Fc domain, such as IgG1 or IgG4 Fc domain. X3 is inserted at H53/E54, N110/K111, L143/T144, Q171/P172, E173/NI74, S186/D187, G188/S189, or G205/N206. The X3 comprises an angiotensin-2 (ang-2) binding peptide sequence, %myostatin% binding peptide sequence, or erythropoietin-mimetic (EPO-mimetic) peptide sequence, thrombopoietin-mimetic (TPO-mimetic) peptide sequence, nerve growth factor (NGF) binding peptide sequence, or B cell activating factor (BAFF) binding peptide sequence. Preferred Host Cell: The host cell is an E. coli cell. Preferred Process: In preparing a pharmacologically active compound, the peptide is selected in a process comprising one or more techniques selected from yeast-based screening, rational design, protein structural analysis, or screening of a phage display library, an E. coli display library, a ribosomal library, or a chemical peptide library. The preparation of the pharmacologic agent is carried out by: preparing a gene construct comprising a nucleic acid sequence encoding an Fc domain wherein a sequence the selected peptide is inserted into or replaces one or more amino acids within the Fc domain; and expressing the gene construct. The gene construct is expressed in an E. coli cell. The protein of interest is a cell surface receptor. The protein of interest has a linear epitope. The protein of interest is a cytokine receptor. USE - The composition of matter is useful for modifying Fc domain that is already linked through an N- or C-terminus or sidechain to a peptide or to a polypeptide.(396 pages)

2/7/72 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2006 The Thomson Corp. All rts. reserv.

0388346 DBR Accession No.: 2006-01842 PATENT
New pharmaceutical preparation comprising Cerberus/Coco derivatives, useful for treating muscular dystrophy, motor neuron disease, inflammatory myopathy, a disease of the neuromuscular junction, or metabolic disease - vector-mediated gene transfer and expression in host cell, monoclonal %antibody%, Fab, humanized %antibody%, chimeric %antibody% with applications in therapy
AUTHOR: KNOPF J; SEEHRA J
PATENT ASSIGNEE: ACCELERON PHARMA INC 2005
PATENT NUMBER: WO 2005115439 PATENT DATE: 20051208 WPI ACCESSION NO.: 2006-020434 (200602)
PRIORITY APPLIC. NO.: US 575062 APPLIC. DATE: 20040527
NATIONAL APPLIC. NO.: WO 2005US18928 APPLIC. DATE: 20050527
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A pharmaceutical preparation comprising a %myostatin% antagonist protein including a %myostatin% binding domain of a Cerberus/Dan/Gremlin polypeptide or its variant, which %myostatin% antagonist protein binds to and neutralizes one or more of nodal and/or %myostatin%, where the pharmaceutical preparation is substantially free of pyrogenic materials for administration as a human or veterinarian therapeutic, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method for inhibiting %myostatin% signal transduction in a muscle cell or an adipose tissue cell in an animal; (2) a method for inducing adipogenic differentiation in an animal; (3) a method for promoting growth of muscle tissue in an animal; (4) a method for treating or preventing congestive heart failure; (5) a method for reducing frailty associated with aging; (6) a method for increasing bone density or accelerating bone fracture repair in a subject; (7) a method for attenuating protein catabolic response in a subject;(8) a method for treating or reducing the severity of a muscular dystrophy, a motor neuron disease, an inflammatory myopathy, a disease of the neuromuscular junction, a myopathy due to endocrine abnormalities, a disease of the peripheral nerve, or a metabolic disease in a patient; and (9) a method for reducing the severity of a pathologic condition which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. BIOTECHNOLOGY - Preferred Preparation: The %myostatin% antagonist protein promotes growth of muscle tissue. The %myostatin% -binding domain is a Cerberus sequence or its variant sequence, or a Coco sequence or its variant sequence. The %myostatin% antagonist protein has diminished potency, relative to a corresponding wild-type Cerberus/Dan/Gremlin polypeptide, for neutralizing BMP-4, where the %myostatin% binding domain is an N-terminally truncated derivative of wild-type Cerberus protein, the derivative comprising a sequence beginning at a position corresponding to any one of residues 106-119 of human Cerberus, or a sequence ending at a position corresponding to any residue after residue 240 of human Cerberus, where the derivative binds %myostatin%, GDF-11, and/or Nodal, but does not substantially bind BMP-4. The %myostatin%-binding domain is encoded by a polynucleotide that hybridizes under high stringency, conditions to the coding sequence for human Cerberus or human Coco. The %myostatin%

antagonist protein binds %myostatin% with a Kd of 1 muM or less. The %myostatin% antagonist protein is a fusion protein including one additional polypeptide portion that enhance one or more of in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. The fusion protein includes an immunoglobulin Fc domain. The fusion protein includes a purification subsequence selected from an epitope tag, a FLAG tag, a polyhistidine sequence, or a GST fusion. The %myostatin% antagonist protein includes one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, or an amino acid conjugated to an organic derivatizing agent. The %myostatin% antagonist protein does not substantially inhibit Activin A signaling in an A204 Reporter Gene Assay. The %myostatin% antagonist protein is a fusion protein that further comprises a second %myostatin% inhibitor domain, which is a polypeptide affinity reagent that selectively binds to %myostatin% and competes with the binding of an ALK7 or ALK4 receptor, where the affinity reagent is an %antibody% agent, e.g. a recombinant %antibody%, a monoclonal %antibody%, a VH domain, a VL domain, an scFv, an Fab fragment, an Fab' fragment, an F(ab')2, an Fv, or a disulfide linked Fv, and where the %antibody% agent is a fully human %antibody% or a humanized chimeric %antibody%, or its antigen binding fragment. The affinity reagent is a peptide or scaffolded peptide that selectively binds to %myostatin% and competes with the binding of an ALK7 or ALK4 receptor. The affinity reagent is a %myostatin% -binding domain of ALK7 or ALK4. The affinity reagent is a small organic molecule that selectively binds to %myostatin% and competes with the binding of an ALK7 or ALK4 receptor. Specifically, the pharmaceutical preparation, for use in a mammal, comprises: (i) a vector including a coding sequence for a %myostatin% antagonist protein described above; (ii) transcriptional control sequences for causing expression of the %myostatin% antagonist protein in vivo in an amount for promoting growth of muscle tissue in the mammal; and (iii) a pharmaceutical carrier. Preferred Method: Inhibiting %myostatin% signal transduction in a muscle cell or an adipose tissue cell in an animal comprises administering the pharmaceutical preparation above. The pharmaceutical preparation is administered in an amount to reduce the severity of a pathologic condition, which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. The pharmaceutical preparation is administered in an amount to prevent, ameliorate or reduce the severity of a wasting disorder. Inducing adipogenic differentiation in an animal comprises administering the pharmaceutical preparation above. The method is used for decreasing body fat proportion in a subject. Promoting growth of muscle tissue in an animal comprises administering the pharmaceutical preparation above. Treating or preventing congestive heart failure comprises administering to a patient an amount of the pharmaceutical preparation above. Reducing frailty associated with aging comprises administering to a patient an amount of the pharmaceutical preparation above. Increasing bone density or accelerating bone fracture repair in a subject comprises administering an amount of the pharmaceutical preparation above. Attenuating protein catabolic response in a subject comprises administering an amount of the pharmaceutical preparation above. Treating or reducing the severity of a muscular dystrophy, a motor neuron disease, an inflammatory myopathy, a disease of the neuromuscular junction, a myopathy due to endocrine abnormalities, a disease of the peripheral nerve, or a metabolic disease in a patient comprises administering an amount of the pharmaceutical preparation above. Reducing the severity of a pathologic condition which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject, comprising treating the subject with a variant Cerberus polypeptide that differs by one or more amino acid residues from a wild-type Cerberus protein, which variant Cerberus polypeptide: (i) binds to and neutralizes one or more of nodal and/or %myostatin%; and (ii) has diminished potency, relative to the wild-type Cerberus protein, for neutraling BMP-4. The method includes co-administration of one or more other compounds to inhibit bone resorption, stimulate bone formation, or increase bone mineral density. The method includes co-administration of a bisphosphonate. The method includes co-administration of one or more other compounds selected from glutamate antagonists, polypeptide growth factors, drugs that increases production of neurotrophic factors, anti-inflammatory agents, caspase inhibitors, protein kinase C inhibitors, vitamin E, coenzyme Q10, or creatine. ACTIVITY - Cardiovascular-Gen; Muscular-Gen; Neuroprotective; Antiinflammatory; Immunosuppressive; Endocrine-Gen; Anorectic; Antidiabetic; Anabolic; Eating-Disorders-Gen; Anti-HIV. No biological data given. MECHANISM OF ACTION - %Myostatin%-Antagonist.Q10 USE - The %myostatin% antagonist protein is useful for preparing a medicament for promoting growth of muscle tissue in a human patient or a non-human mammal (claimed). The preparation and methods are useful for inhibiting %myostatin% signal transduction in a muscle cell or an adipose tissue cell in an animal, for inducing adipogenic differentiation in an animal, for promoting growth of muscle tissue in an animal, for treating or preventing congestive heart failure, for reducing frailty associated with aging, for increasing bone density or accelerating bone fracture repair in a subject, for attenuating protein catabolic response in a subject, and for reducing the severity of a pathologic condition which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. The preparation and methods are useful for treating or reducing the severity of muscular dystrophy (e.g. Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Emery-Dreifuss Muscular Dystrophy (EDMD), Limb-Girdle Muscular Dystrophy (LGMD), Facioscapulohumeral Muscular Dystrophy (FSH or FSHD) (Also known as Landouzy-Dejerine), Myotonie Dystrophy (MMD) (Also known as Steinert's Disease), Oculopharyngeal Muscular Dystrophy (OPMD), Distal Muscular Dystrophy (DD), Congenital Muscular Dystrophy (CMD), Myotonia Congenita (MC), Paramyotonia Congenita (PC), Central Core Disease (CCD), Nemaline Myopathy (NM), Myotubular Myopathy (MTM or MM),or Periodic Paralysis (PP)), motor neuron disease (e.g. myotrophic Lateral Sclerosis (ALS) (Also known as Lou Gehrig's Disease), Infantile Progressive Spinal Muscular Atrophy (SMA, SMAI or WH) (Also known as SMA Type 1, Werdnig-Hoffman), Intermediate Spinal Muscular Atrophy (SMA or SMA2) (Also known as SMA Type 2), Juvenile Spinal Muscular Atrophy(SMA, SMA3 or KW) (Also known as SMA Type 3, Kugelberg-Welander), Spinal Bulbar Muscular Atrophy (SBMA) (Also known as Kennedy's Disease and X-Linked SBMA), or Adult Spinal Muscular Atrophy (SMA)), inflammatory myopathy (e.g. Dermatomyositis (PM/DM), Polymyositis (PM/DM), or Inclusion Body Myositis (IBM)), disease of the neuromuscular junction (e.g. Myasthenia Gravis (MG), Lambert-Eaton Syndrome (LES), or Congenital Myasthenic Syndrome (CMS)), myopathy due to endocrine abnormalities (e.g. Hyperthyroid Myopathy (HYPTM), or Hypothyroid Myopathy (HYPOTM)), disease of peripheral nerve (e.g. Charcot-Marie-Tooth Disease (CMT), Dejerine-Sottas Disease (DS), or Friedreich's Ataxia (FA)), metabolic disease (e.g. Phosphorylase Deficiency (MPD or PYGM), Acid Maltase Deficiency (AMD), Phosphofructokinase Deficiency (PFKM), Debrancher Enzyme Deficiency (DBD), Mitochondrial Myopathy (MITO), Carnitine Deficiency (CD), Carnitine Palmityl Transferase Deficiency (CPT), Phosphoglycerate Kinase Deficiency (PGK), Phosphoglycerate Mutase Deficiency (PGAM or PGAMM), Lactate Dehydrogenase Deficiency (LDHA), Myoadenylate Deaminase Deficiency (MAD), obesity, or type II diabetes), or wasting disorder (e.g. age-related wasting, cachexia, anorexia, DMD syndrome, BMD syndrome, AIDS wasting syndrome, muscular dystrophies, or neuromuscular diseases). ADMINISTRATION - Dosage is 0.0001-100 mg/kg, by oral, intravesical, nasal, rectal, intravaginal, parenteral, intracisternal, topical, buccal, or sublingual means. EXAMPLE - No relevant example given.(52 pages)

2/7/73 (Item 7 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0383683 DBR Accession No.: 2005-29389 PATENT
New anti-%myostatin% monoclonal %antibodies%, useful for increasing muscle mass, increasing bone density, or treating disorders, e.g. cachexia, myopathy, muscular dystrophy, osteoporosis, cardiac failure, or type II diabetes - involving vector-mediated gene transfer and expression in

host cell for use in therapy
AUTHOR: SMITH R C; KIKLY K K; TOBIAS L O; HAN B
PATENT ASSIGNEE: LILLY and CO ELI 2005
PATENT NUMBER: WO 200594446 PATENT DATE: 20051013 WPI ACCESSION NO.: 2005-690154 (200571)
PRIORITY APPLIC. NO.: US 559621 APPLIC. DATE: 20040405
NATIONAL APPLIC. NO.: WO 2005US9307 APPLIC. DATE: 20050317
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An anti-%myostatin% monoclonal %antibody% comprises two polypeptides comprising fully defined 109-124 amino acid sequences (SEQ ID NO. 3-17) given in the specification, is new. The polypeptide sequences are selected from SEQ ID NO. 3 and 12, 4 and 13, 3 and 14, 5 and 12, 6 and 15, 7 and 17, 8 and 12, 9 and 16, 10 and 12, or 11 and 12. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a process of producing an anti-%myostatin% monoclonal %antibody%; (2) a monoclonal %antibody% produced by the process of (1); (3) a pharmaceutical composition comprising the %antibody% above; (4) a method of increasing muscle mass; and (5) a method of treating or preventing frailty, cachexia, muscle wasting, muscle weakness, myopathy, muscular dystrophy, osteoporosis, COPD, renal failure or disease, liver failure or disease, cardiac failure, type II diabetes, or metabolic syndrome. BIOTECHNOLOGY - Preparation (claimed): Producing an anti-%myostatin% monoclonal %antibody% comprises: (A) immunizing a non-human animal by injecting with a peptide selected from (i) an immunogenic peptide comprising fully defined 25 amino acid sequences (SEQ ID NO. 46 or 43), (ii) an immunogenic peptide comprising 5-24 contiguous amino acids of SEQ ID NO. 46 or 43, where at least one amino acid differs from that in GDF-11 at the equivalent position, (iii) an immunogenic peptide comprising amino acids 40-64 of mature %myostatin% of any mammal, or (iv) an immunogenic peptide comprising 5-24 contiguous amino acids of the peptide comprising amino acids 40-64 of mature %myostatin% of any mammal, where at least one amino acid differs from that in GDF-11 at the equivalent position; (B) generating anti-%myostatin% monoclonal %antibodies% from the immunized animal; and (C) screening the anti-%myostatin% monoclonal %antibodies% generated for %antibodies% that specifically bind mature %myostatin% or its portion comprising the immunogenic peptide, or the immunogenic peptide. Alternatively, producing an anti-%myostatin% monoclonal %antibody% comprises: (A) immunizing a non-human animal by injecting with a peptide selected from (i) an immunogenic peptide comprising fully defined 25 amino acid sequences (SEQ ID NO. 46 or 43), (ii) an immunogenic peptide comprising 5-24 contiguous amino acids of SEQ ID NO. 46 or 43, where at least one amino acid differs from that in GDF-11 at the equivalent position, (iii) an immunogenic peptide comprising amino acids 40-64 of mature %myostatin% of any mammal, or (iv) an immunogenic peptide comprising 5-24 contiguous amino acids of the peptide comprising amino acids 40-64 of mature %myostatin% of any mammal, where at least one amino acid differs from that in GDF-11 at the equivalent position; (B) generating anti-%myostatin% monoclonal %antibodies% from the immunized animal; and (C) screening the anti-%myostatin% monoclonal %antibodies% generated for %antibodies% that specifically bind a peptide selected from (i) an immunogenic peptide comprising fully defined 25 amino acid sequences (SEQ ID NO. 46 or 43), (ii) an immunogenic peptide comprising 5-24 contiguous amino acids of SEQ ID NO. 46 or 43, where at least one amino acid differs from that in GDF-11 at the equivalent position, (iii) an immunogenic peptide comprising amino acids 40-64 of mature %myostatin% of any mammal, or (iv) an immunogenic peptide comprising 5-24 contiguous amino acids of the peptide comprising amino acids 40-64 of mature %myostatin% of any mammal, where at least one amino acid differs from that in GDF-11 at the equivalent position. Preferred %Antibody%: Specifically, the anti-%myostatin% monoclonal %antibody% comprises a LCVR (light chain variable region) comprising 1, 2, or 3 peptides selected from (a) a peptide at CDR1 (complementarity determining region) with a fully defined 10 amino acid sequence (SEQ ID NO. 38), (b) a peptide at CDR2 with a fully defined 7 amino acid sequence (SEQ ID NO. 23), or (c) a peptide at CDR3 with a fully defined 9 amino acid sequence (SEQ ID NO. 56). Alternatively, the anti-%myostatin% monoclonal %antibody% also comprises a HCVR comprising 1, 2, or 3 peptides selected from: (a) a peptide at CDR1 with a fully defined 12 amino acid sequence (SEQ ID NO. 55), (b) a peptide at CDR2 with a fully defined 16 amino acid sequence (SEQ ID NO. 41), or (c) a peptide at CDR3 with a fully defined 14 amino acid sequence (SEQ ID NO. 42). The LCVR comprises 1, 2, or 3 peptides selected from: (a) a peptide at LCVR CDR1 with a sequence comprising fully defined 10 amino acid sequences (SEQ ID NO. 18-22); (b) a peptide at LCVR CDR2 with a sequence comprising fully defined 7 amino acid sequence (SEQ ID NO. 23); or (c) a peptide at LCVR CDR3 with a sequence comprising fully defined 9 amino acid sequences (SEQ ID NO. 24-28). The HCVR comprises 1, 2, or 3 peptides selected from: (a) a peptide at HCVR CDR1 with a sequence comprising fully defined 12 amino acid sequences (SEQ ID NO. 29-31, 47-54); (b) a peptide at HCVR CDR2 with a sequence comprising fully defined 16 amino acid sequences (SEQ ID NO. 32-35); or (c) a peptide at HCVR CDR3 with a sequence comprising fully defined 14 amino acid sequences (SEQ ID NO. 36 or 37). The monoclonal %antibody% is a full-length %antibody%, a substantially intact %antibody%, a chimeric %antibody% , a Fab fragment, an F(ab')2 fragment, or a single chain Fv fragment. Preferably, the monoclonal %antibody% is a humanized %antibody%. The constant region present in the %antibody% originates from the genome of an animal selected from domestic animals, sports animals, or food-source animals. Preferred Composition: The pharmaceutical composition further comprises a carrier. Preferred Method: Increasing muscle mass comprises administering to a subject in need an amount of the pharmaceutical composition above. Treating or preventing frailty, cachexia, muscle wasting, muscle weakness, myopathy, muscular dystrophy, osteoporosis, COPD, renal failure or disease, liver failure or disease, cardiac failure, type II diabetes, or metabolic syndrome comprises administering to a subject in need an amount of the pharmaceutical composition above. ACTIVITY - Anabolic; Immunomodulator; Muscular-Gen.; Osteopathic; Respiratory-Gen.; Nephrotropic; Hepatotropic; Cardiant; Antidiabetic. No biological data given. MECHANISM OF ACTION - GDF-8 modulator; %Myostatin% modulator. USE - The anti-%myostatin% monoclonal %antibodies% are useful for increasing muscle mass, increasing bone density, or for treating various disorders in mammals, including frailty, cachexia, muscle wasting, muscle weakness, myopathy, muscular dystrophy, osteoporosis, COPD (Chronic Obstructive Pulmonary Disease), renal failure or disease, liver failure or disease, cardiac failure, type II diabetes, or metabolic syndrome. ADMINISTRATION - Dosage is 0.1 micrograms/kg - 100 mg/kg of total body weight. Administration can be through oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository route. EXAMPLE - Clones of anti-myosin Fabs were isolated from a library created by immunizing C57B1/6 wild-type mice using Omniclonal (TRM) %antibody% technology. The mice were immunized with an immunogenic polypeptide. The sequence is identical to the sequence spanning amino acids 40-64 of the mature form of human %myostatin%. To improve the immunogenicity of this peptide, the carrier protein, keyhole limpet hemocyanin, and a helper T-cell peptide were conjugated to the immunogenic peptide. The HCVR and LCVR and framework amino acid sequences were identified as the sequences of Fabs from the library, which bind mature %myostatin% and bind the immunogenic peptide and neutralize a %myostatin% activity.(84 pages)

2/7/74 (Item 8 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0381493 DBR Accession No.: 2005-27199 PATENT
Pharmaceutical preparation for inhibiting %myostatin%, treating muscular dystrophy e.g. myotonic dystrophy, obesity, type II diabetes, congestive heart failure, has %myostatin% inhibitor that inhibit binding of ALK7 receptor to %myostatin% - %myostatin% inhibitor and receptor fusion protein for use in disease therapy
AUTHOR: KNOPF J; SEEHRA J
PATENT ASSIGNEE: ACCELERON PHARMA INC 2005
PATENT NUMBER: WO 200584699 PATENT DATE: 20050915 WPI ACCESSION NO.: 2005-649432 (200566)
PRIORITY APPLIC. NO.: US 549352 APPLIC. DATE: 20040302

NATIONAL APPLIC. NO.: WO 2005US7281 APPLIC. DATE: 20050302
LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Pharmaceutical preparation (I) for inhibiting %myostatin%, comprises a %myostatin% inhibitor that binds to %myostatin%, to inhibit binding of an ALK7 receptor to %myostatin%, where the preparation is substantially free of pyrogenic materials so as to be suitable for administration to an mammal. DETAILED DESCRIPTION - Pharmaceutical preparation (I) for inhibiting %myostatin%, comprises a %myostatin% inhibitor that binds to %myostatin%, to inhibit binding of an ALK7 receptor to %myostatin% , where the preparation is substantially free of pyrogenic materials so as to be suitable for administration to an mammal. Pharmaceutical preparation (II) for promoting growth of muscle tissue in a mammal, comprises a polypeptide that comprises a ligand binding domain of an ALK7 receptor, where the preparation is substantially free of pyrogenic materials so as to be suitable for administration to an mammal. Pharmaceutical preparation (III) suitable for use in a mammal, comprises a vector including a coding sequence for polypeptide %myostatin% inhibitor that binds to an ALK7 receptor binding site on %myostatin% and inhibits signaling by %myostatin% , and transcriptional control sequences for causing expression of the polypeptide %myostatin% inhibitor in vivo, for promoting growth of muscle tissue in the mammal, and a carrier. INDEPENDENT CLAIMS are also included for: (1) a packaged pharmaceutical (IV) comprising (I), (II) or (III) and labeled for use in promoting growth of muscle tissue in a human patient, and for vetemerian use in promoting growth of muscle tissue in a non-human mammal; and (2) reducing (M1) the severity of a pathologic condition, which is characterized at least in part by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject, involves treating the subject with a soluble polypeptide including a ligand binding domain from the extracellular domain of ALK7. BIOTECHNOLOGY - Preferred Preparation: In (I), the mysotatin inhibitor is a polypeptide that includes a %myostatin% binding domain of an ALK7 receptor. The mysotatin inhibitor is a soluble ALK7 receptor. The soluble ALK7 receptor has a fully defined 413 or 336 amino acid (SEQ ID No. 4 or 6) sequence given in the specification. The %myostatin% binding domains binds %myostatin% with a Kd of 1 muM or less. The polypeptide is a fusion protein including, in addition to the %myostatin% binding domain, enhance one or more of in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes and/or purification. The fusion protein includes an immunoglobulin Fc domain. The fusion protein includes a purification subsequence chosen from epitope tag, FLAG tag, polyhistidine sequence, and GST fusion. The polypeptide includes one or more modified amino acid residues chosen from glycosylated amino acid, polyethylene glycosylated amino acid, farnesylated amino acid, acetylated amino acid, biotinylated amino acid, amino acid conjugated to a lipid moiety, and amino acid conjugated to an organic derivatizing agent. The mysotatin inhibitor has dissociation constant (Kd) for %myostatin% binding that is at least 2 times less than its Kd for binding GDF11. The mysotatin inhibitor is a polypeptide affinity reagent that selectively binds to %myostatin% and competes with the binding of an ALK7 receptor, where the affinity reagent is an %antibody% agent. The %antibody% agent is a recombinant %antibody%, monoclonal %antibody%, V H domain, V L domain, scFv, Fab fragment, Fab' fragment, F(ab')2, Fv, or disulfide linked Fv. The %antibody% agent is a fully human %antibody% or a humanized chimeric %antibody%, or its antigen binding fragment. The affinity reagent is a peptide or scaffolded peptide that selectively binds to %myostatin% and competes with the binding of an ALK7 receptor. The mysotatin inhibitor is a small organic molecule that selectively binds to %myostatin% and competes with the binding of an ALK7 receptor. In (II), the domain of an ALK7 receptor domain has a fully defined 493 amino acid (SEQ ID No. 2) sequence given in the specification, or its variant that retains %myostatin% binding activity. The %myostatin% binding domain has 26-100 amino acid residues of SEQ ID No. 2, or its variant that retains %myostatin% binding activity. Preferred Method: In (M1), the co-administration of one or more other compounds that inhibit bone resorption, stimulate bone formation, and increase bone mineral density. (M1) involves co-administration of a bisphosphonate. The co-administration of one or more other compounds are chosen from glutamate antagonists, polypeptide growth factors, drugs that increases production of neurotrophic factors, anti-inflammatory agents, caspase inhibitors, protein kinase C inhibitors, vitamin E, coenzyme Q10, and creatine. The %myostatin% binding domain includes amino acid residues such as Leu-Lys-Cys-Val-Cys-Leu-Leu-Cys-Asp-Ser-Ser-Asn-Phe-Thr-Cys-Gln-Thr-Glu -Gly-Ala-Cys-Trp-Ala-Ser-Val-Met-Leu-Thr-Asn-Gly-Lys-Glu-Gln-Val-Ile-Ly s-Ser-Cys-Val-Ser-Leu-Pro-Glu-Leu-Asn-Ala-Gln-Val-Phe-Cys-His-Ser-Ser-A sn-Asn-Val-Thr-Lys-Thr-Glu-Cys-Cys-Phe-Thr-Asp-Phe-Cys-Asn-Asn-Ile-Thr-Leu-His-Leu-Pro (SEQ ID No. 2). ACTIVITY - Muscular-Gen; Immunomodulator; Eating-Disorder-Gen; Anorectic; Antidiabetic; Anti-HIV; Osteopathic; Cardiovascular-Gen; CNS-Gen; Neuroprotective; Antiinflammatory; Anabolic; Dermatological. In vivo analysis of soluble ALK7 that increases muscle mass was carried out as follows. The male CB-17 SCID mice (6 week old) were administered with either ALK7-Fc (test) or phosphate buffered saline (PBS) (control) by intraperitoneal injection. Each animal was received a total of 5 injections, by days 0, 4, 8, 15, and 22. Individual animal weights were taken once per week. Mice were monitored daily for signs of toxicity and morbidity. All mice were euthanized on twenty-eight days after initiation of test or control administration. Then, the mice were euthanized by carbon dioxide inhalation and the gastrocnemius, femoris rectus (quadriceps) and diaphragm muscles were dissected and weighed. Results indicated that the dosage of 3 mg/kg caused a statistically significant increase in muscle mass relative to control, and in the case of gastrocnemius and diaphragm muscles, the lower dosage of 1 mg/kg also caused statistically significant increase in muscle mass. MECHANISM OF ACTION - Inhibits binding of an ALK7 receptor to %myostatin% (claimed). USE - (I), (II) or (III) is useful for inhibiting %myostatin% signal transduction in a muscle cell or adipose tissue cell in an animal, and to reduce the severity of a pathologic condition, which is characterized at least in portion by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. (I), (II) or (III) is useful for preventing, ameliorating or reducing the severity of a wasting disorder (such as cachexia, anorexia, Duchenne muscular dystrophy (DMD) syndrome, Becker muscular dystrophy (BMD) syndrome, AIDS wasting syndrome, muscular dystrophies and neuromuscular diseases), metabolic disorder (obesity and type II diabetes). (I), (II) or (III) is useful for inducing adipogenic differentiation in an animal, which is useful for decreasing body fat proportion in a subject. (I), (II) or (III) is useful for promoting growth of muscle tissue in an animal. (I), (II) or (III) is useful for treating or preventing congestive heart failure and for reducing frailty associated with aging. (I), (II) or (III) is useful for increasing bone density or accelerating bone fracture repair and for attenuating protein catabolic response in a subject. (I), (II) or (III) is useful for treating or reducing the severity of a muscular dystrophy in a patient. The muscular dystrophy includes DMD, BMD, Emery-Dreifuss muscular dystrophy (EDMD), Limb-Girdle muscular dystrophy (LGMD), Facioscapulohumeral muscular dystrophy (FSH or FSHD) (also known as Landouzy-Dejerine), myotonic dystrophy (MMD) (also known as Steinert's disease), oculopharynageal muscular dystrophy (OPMD), distal muscular dystrophy (DD), congenital muscular dystrophy (CMD), myotonia congenita (MC), paramyotonia congenita (PC), central core disease (CCD), nemaline myopathy (NM), myotubular myopathy (MTM or MM), and periodic paralysis (PP), (I), (II) or (III) is useful for treating or reducing the severity of a motor neuron disease in a patient. The motor neuron disease includes amyotrophic lateral sclerosis (ALS) (also known as Lou Gehrig's Disease), infantile progressive spinal muscular atrophy (SMA, SMA1 or WH) (also known as SMA Type 1, Werding-Hoffman), intermediate spinal muscular atrophy (SMA or SMA2) (also known as SMA Type 2), juvenile spinal muscular atrophy (SMA, SMA3 or KW) (also known as SMA Type 3, Kugelberg-welander), spinal bulbar muscular atrophy (SBMA) (also known as Kennedy's disease and X-Linked SBMA), and adult spinal muscular atrophy (SMA). (I), (II) or (III) is useful for treating or reducing the severity of a inflammatory myopathy in a patient. The inflammatory myopathy is chosen from dermatomyositis (PM/DM), polymyositis (PM/DM), and inclusion body myositis (IBM), and for treating or reducing the severity of a disease of the neuromuscular junction in a patient. The neuromuscular junction disease is chosen

from myasthenia gravis, Lambert-Eaton Syndrome (LES), and congenital myasthenic syndrome (CMS). (I), (II) or (III) is useful for treating or reducing the severity of a myopathy, peripheral nerve disease, and metabolic disease (all claimed). ADMINISTRATION - (I), (II) or (III) is administered at a dose of 0.0001-100 mg/kg/day, by oral, intravenous, intravesical, intraarterial, intraperitoneal, local, intramuscular, intrathecal, intracapsular, intraorbital, intracardiac, subcutaneous, subcuticular, or intradermal route. EXAMPLE - No relevant example is given.(57 pages)

2/7/75 (Item 9 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0373101 DBR Accession No.: 2005-18807 PATENT
New polypeptides involved in the regulation of muscle growth, useful for regulating or promoting muscle growth and for treating or preventing conditions associated with muscle growth or muscle wasting - recombinant protein production and antisense sequence for use in disease therapy and gene therapy
AUTHOR: SHARMA M; BERRY C; THOMAS M; KAMBADUR R; BOWER R S
PATENT ASSIGNEE: OVITA LTD 2005
PATENT NUMBER: WO 200551993 PATENT DATE: 20050609 WPI ACCESSION NO.: 2005-405354 (200541)
PRIORITY APPLIC. NO.: NZ 203Z-529860 APPLIC. DATE: 20031128
NATIONAL APPLIC. NO.: WO 2004NZ308 APPLIC. DATE: 20041126
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A polypeptide comprising a sequence of 192 amino acids fully defined in the specification (SEQ ID NO: 2 or 4), is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a polynucleotide that encodes the above polypeptide; (2) a fusion protein comprising the above polypeptide or its fragment; (3) a vector comprising the polynucleotide; or in the 5'-3' direction: a gene promoter sequence, the above polynucleotide sequence, and a gene termination sequence; (4) a host cell containing the vector; (5) a composition for regulating muscle growth or for modulating mighty gene expression; (6) regulating muscle growth of an organism; (7) a transgenic animal comprising the above vector or composition; (8) predicting muscle mass in an animal; (9) detecting a variant of mighty; (10) breeding an animal having improved muscle mass; (11) an %antibody% that preferentially binds a polypeptide comprising SEQ ID NO: 2 or 4 or a polypeptide having at least 95, 90 or 70 % sequence identity to SEQ ID NO: 2 or 4; (12) screening for one or more compounds that are potentially useful in inhibiting or promoting muscle growth; and (13) expressing a desired protein in a muscle cell. BIOTECHNOLOGY - Preferred Polypeptide: The polypeptide is encoded by the polynucleotide as mentioned above. Preferred Polynucleotide: The polynucleotide comprises: (a) a sequence of 576 bp (SEQ ID NO: 1 or 3) or 2071 bp (SEQ ID NO: 5) fully defined in the specification; (b) complements, reverse complements or reverse sequences of SEQ ID NO: 1 or 3; (c) a nucleotide sequence that differs from SEQ ID NO: 1 or 3 as a result of silent substitution(s) or substitution(s) that result(s) in conservative substitution(s) in the resulting amino acid; (d) a polynucleotide sequence having at least 95, 90 or 70% identity to SEQ ID NO: 5; or (e) a fragment or variant of SEQ ID NO: 5 having promoter activity. The isolated polynucleotide comprises at least the 200 nucleotides upstream of the mighty initiation site. It comprises fragments of any one of 209, 287, 315, 400, 600, 1000 and 2100 nucleotides upstream of the mighty initiation site. Preferred Vector: The polynucleotide is in a sense or antisense orientation. Preferred Composition: The composition for regulating muscle growth comprises: (a) a polynucleotide comprising SEQ ID NO: 1, 3 or 5; (b) a polynucleotide having at least 95, 90 or 70 % sequence identity to (a); (c) a fragment or variant of (a) or (b); (d) a complement, reverse complement or an antisense polynucleotide of any of (a)-(c); (e) a polypeptide encoded by (a)-(c); (f) a polypeptide comprising SEQ ID NO: 2 or 4; (g) a fragment or variant of (e) or (f); or (h) a polypeptide having at least 95, 90 or 70 % sequence identity to (e) or (f). The composition for modulating mighty gene expression comprises a compound capable of binding to the above-mentioned polynucleotide or its complement, reverse complement, fragment or variant. The compound is an anti-sense polynucleotide or an interfering RNA molecule. The interfering RNA molecule is an RNAi or siRNA molecule. The compound may also be %myostatin% or a %myostatin% mimetic. The %myostatin% mimetic is a %myostatin% peptide C-terminally truncated at or between amino acid positions 330 and 350, or at any one of amino acid positions 330, 335 and 350. The compound may also be an %antibody% . Preferred Transgenic Animal: The transgenic animal has an increased muscle mass. It is selected from sheep, cow, bull, deer, poultry, turkey, pig, horse, mouse, rat or human. Preferred Method: Regulating muscle growth of an organism comprises administering to the organism the composition as mentioned above. Predicting muscle mass in an animal comprises: (a) obtaining a sample from the animal; (b) determining the gene expression level from a polynucleotide having SEQ ID NO: 1 or 3, a polynucleotide having at least 95, 90 or 70 % sequence identity to SEQ ID NO: 1 or 3, or a fragment or variant of the polynucleotide; or determining the amount of a polypeptide having SEQ ID NO: 2 or 4, a polypeptide having at least 95, 90 or 70 % sequence identity to SEQ ID NO: 2 or 4, or a fragment or variant of the polypeptide; (c) comparing the gene expression level or amount of polypeptide to an average; and (d) predicting the muscle mass of the animal. The level of gene expression is determined using RT-PCR or northern analysis. The amount of the polypeptide is determined using ELISA or Western blot analysis. Detecting a variant of mighty comprises using the above-mentioned polynucleotide or its complement, reverse complement, fragment or variant, to screen a sample from an organism for the variant of mighty. The variant is a polymorphism, particularly a single nucleotide polymorphism. The variant of mighty is associated with an altered muscle phenotype. Breeding an animal having improved muscle mass comprises selecting one or more animals predicted to have an increase in muscle mass using the above methods, and breeding the animals predicted to have an increased muscle mass to produce an animal having an improved muscle mass. The animal is a sheep, cow, bull, deer, poultry, turkey, pig, horse, mouse, rat, fish or human. Screening for one or more compounds that are potentially useful in inhibiting or promoting muscle growth comprises inserting the above polynucleotide into a vector linked to a marker gene; transforming a host cell with the vector; administering a compound to the host cell; and determining any difference in the level of the marker gene expression. The vector is a prokaryotic plasmid, a eukaryotic plasmid or a viral vector. The marker gene is a polynucleotide that encodes a green fluorescent protein, a red fluorescent protein, a luciferase enzyme, or a beta-galactosidase enzyme. Expressing a desired protein in a muscle cell comprises isolating a polynucleotide sequence that encodes the gene to be expressed; inserting the polynucleotide operably linked to the polynucleotide sequence of the protein to be expressed in a 5'-3' orientation into a vector; and introducing the vector into a muscle host cell. The vector is a eukaryotic vector, a viral vector, or any vector suitable for gene therapy. The host cell is a primary myoblast cell line, a transformed myoblast cell line or any cell line in which the mighty promoter is active. The host cell is an in vivo skeletal or cardiac muscle cell of a host animal. The host animal is a sheep, cow, deer, bull, poultry, turkey, pig, horse, mouse, rat, fish or human. ACTIVITY - Muscular-Gen.; Neuroprotective; Cardiant; Anabolic. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The antigenic fragment of the polypeptide is used in the production of an %antibody% that preferentially binds the above polypeptide. The composition (including the polypeptide and polynucleotide) and methods are useful for the treatment or prophylaxis of a disease associated with muscle growth or muscle atrophy, such as muscular dystrophy, muscle cachexia, atrophy, hypertrophy, muscle wasting associated with cancer or HIV, amyotrophic lateral sclerosis, or diseases associated with cardiac muscle growth, including infarct. These may also be used for promoting muscle regeneration after muscle injury, for producing an animal having increased muscle mass, or for producing a medicament for the regulation of muscle growth or for the treatment or prophylaxis of such diseases (all claimed). EXAMPLE - No relevant example given. (84 pages)

2/7/76 (Item 10 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0368871 DBR Accession No.: 2005-14577 PATENT
New muscle-targeting fusion protein for treating muscle-related diseases, e.g. muscle atrophy, comprises a ligand that binds a target surface protein; a therapeutic agent; and a multimerizing component and/or a signal sequence - muscle-targeting fusion protein gene transfer and expression in host cell for muscle-related condition or disease gene therapy
AUTHOR: GLASS D J
PATENT ASSIGNEE: REGENERON PHARM INC 2005
PATENT NUMBER: WO 200533134 PATENT DATE: 20050414 WPI ACCESSION NO.: 2005-285404 (200529)
PRIORITY APPLIC. NO.: US 584956 APPLIC. DATE: 20040702
NATIONAL APPLIC. NO.: WO 2004US32233 APPLIC. DATE: 20040930
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A targeting fusion polypeptide comprising a first component comprising a targeting ligand, or its derivative or fragment, capable of binding specifically to a pre-selected cell surface protein; a second component comprising at least one active or therapeutic agent; and optionally, a multimerizing component capable of forming a multimer with another targeting fusion polypeptide; and/or a signal sequence, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid molecule encoding the above fusion polypeptide; and (2) a pharmaceutical composition comprising the above fusion polypeptide and a carrier. WIDER DISCLOSURE - Also disclosed are: (a) a vector comprising the above nucleic acid molecule; (b) a method of producing the above fusion polypeptide; (c) a method of treating a disease or condition; and (d) a method for activating and/or phosphorylating multiple cell receptors simultaneously. BIOTECHNOLOGY - Preferred Fusion Polypeptide: The fusion polypeptide is a muscle-targeting fusion polypeptide, and the cell surface protein is a muscle cell surface protein. The targeting fusion polypeptide further comprises a second active or therapeutic agent. The targeting ligand comprises 3 N-terminal cadherin domains of human M-cadherin. The muscle surface protein is a receptor. The receptor is MuSK and the targeting ligand is agrin or a fragment of agrin capable of binding the MuSK receptor, or an anti-MuSK %antibody% or its mutant, fragment or derivative having biological activity. The fusion polypeptide may be a combined therapeutic fusion polypeptide, which comprises a first active or therapeutic agent, and a second active or therapeutic agent; and optionally, a multimerizing component capable of forming a multimer with another therapeutic fusion polypeptide, and/or a signal sequence. The first or second active or therapeutic agent is an activating %antibody% or a blocking %antibody%. The blocking %antibody% blocks a component selected from %myostatin%, activin receptor, BMP receptor 1, TNF receptor and IL-1 receptor. The activating %antibody% activates a component selected from IGF1 receptor, B2-adrenergic receptor and the IL-15 receptor complex. The first and/or second active or therapeutic agent is a ligand for a cell surface receptor, and is capable of binding and activating the cell surface receptor. The active or therapeutic agent is selected from IL-15, myotrophin, urocortin, urocortin II, a natural or mutant human IGF1 or IGF2, insulin, human %myostatin% propeptide, hGH, proliferin, follistatin, FSTL1, and FLRG, or its mutant, derivative or fragment having biologically activity. The mutant human IGF1 comprises a modification selected from delGPE (1-3), E3R and E3A; and a modification selected from R36A, R37A, del36 and del37. The first active or therapeutic agent is hGH and the second active or therapeutic agent is the mutant human IGF1 as mentioned above. The fusion polypeptide may be IGF1-related fusion polypeptide comprising the human IGF1 comprising the modification mentioned above; a multimerizing component capable of forming a multimer with another IGF-related polypeptide; and optionally, a signal sequence. Alternatively, the fusion polypeptide is a %myostatin% inhibiting fusion polypeptide comprising (P)x-M, P = human %myostatin% propeptide, or its fragment or variant, capable of binding and inhibiting the biological activity of %myostatin%; M = multimerizing component; and X = 1-10. The multimerizing component of the targeting fusion polypeptide is selected from a multimerizing component comprising a cleavable region (C-region); a truncated multimerizing component; an amino acid sequence of 1-500 amino acids in length, optionally comprising at least one cysteine residue; a leucine zipper; a helix loop motif; a coil-coil motif; and an Fc- protein. The first component targeting ligand comprises 3 cadherin domains of a human cadherin. The human cadherin is selected from muscle cadherin (M-cadherin), nerve cadherin (N-cadherin), vascular endothelial cadherin (VE-cadherin), cadherin 17 (liver-intestine cadherin), cadherin 13 (heart cadherin), cadherin 26, cadherin 24 and epithelial cadherin (E-cadherin or cadherin-1). The first component may comprise 3 cadherin domains of human VE-cadherin. The second component active or therapeutic agent is vascular endothelial growth factor (VEGF), angiopoietin-1, angiopoietin-2, or their mutants, derivatives or fragments, having biological activity. The multimeric fusion polypeptide comprises 2 or more of the fusion polypeptides mentioned above. ACTIVITY - Muscular-Gen. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The fusion protein is useful in therapy or in the manufacture of a medicament for the treatment of a muscle-related condition or disease in a subject suffering from a condition which would benefit from the medicament, such as muscle atrophy or muscle dystrophy (claimed). ADMINISTRATION - Dosages may range from about 0.01 pg/kg-1 mg/kg of body weight. Administration can be intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, pulmonary, intranasal, intraocular, epidural, topical or oral. EXAMPLE - No relevant example given. (84 pages)

2/7/77 (Item 11 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0342726 DBR Accession No.: 2004-15018 PATENT
Novel synthetic promoter functional in fish cell, useful for driving expression of disease resistance genes in fish such as catfish - vector-mediated cecropin-B, preprocecropin-B or cecropin-P1 gene transfer and expression in fish cell for transgenic catfish construction
AUTHOR: DUNHAM R A; LIU Z; WARR G W
PATENT ASSIGNEE: UNIV AUBURN; MUSC FOUND RES DEV 2004
PATENT NUMBER: WO 200444145 PATENT DATE: 20040527 WPI ACCESSION NO.: 2004-420297 (200439)
PRIORITY APPLIC. NO.: US 702395 APPLIC. DATE: 20031105
NATIONAL APPLIC. NO.: WO 2003US35263 APPLIC. DATE: 20031106
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A synthetic promoter (I) that is functional in a fish cell, comprising a nucleotide chosen from nucleotide sequence having a fully defined sequence (S1) of 161 base pairs or 207 base pairs as given in the specification, or a nucleotide sequence with 90%, 80% or at least 70% sequence identity to (S1), is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an expression cassette (II) comprising: (a) synthetic promoter which is operably linked in proper reading frame to a nucleotide sequence of interest; or (b) a promoter that is functional in a fish cell, where the promoter is chosen from (I), a carp beta-actin promoter comprising the nucleotide sequence having a fully defined sequence of 1571 base pairs as given in the specification, a channel catfish %myostatin% promoter comprising the nucleotide sequence having a fully defined sequence of 1586 base pairs as given in the specification, channel catfish alpha-actin promoter comprising the nucleotide sequence having a fully defined sequence of 1208 base pairs as given in the specification, channel catfish creatine kinase promoter comprising the nucleotide sequence having a fully defined sequence of 1799 base pairs as given in the specification, a salmon metallothionein promoter comprising a nucleotide sequence having a fully defined sequence of 272 base pairs as given in the specification, a salmon histone H3 promoter comprising the nucleotide sequence having a fully defined sequence of 470 base pairs as given in the specification, and a promoter comprising a nucleotide sequence with at least 70% sequence identity to the above

mentioned sequences, where the promoter is operably linked in proper reading frame to a nucleotide sequence encoding an anti-pathogenic polypeptide of interest, which is chosen from mature form of a cecropin polypeptide, a prepro-form of the cecropin polypeptide, pro-form of the cecropin polypeptide, a fusion polypeptide comprising a catfish immunoglobulin variable heavy chain (Ig Vh) leader and the pro-form of the cecropin polypeptide, a fusion polypeptide comprising a catfish Ig Vh leader and the mature form of the cecropin polypeptide, and a polypeptide having anti-pathogenic activity and at least 70% sequence identity to the above mentioned polypeptides; (2) a vector comprising (II); (3) a host cell having (II) which is stably incorporated in its genome; (4) a fish egg having (II) which is stably incorporated in its genome; (5) a transgenic fish (III) having (II) which is stably incorporated in its genome; and (6) transgenic catfish farming, involves breeding the catfish produced using (II) to yield transgenic offspring. WIDER DISCLOSURE - A composition comprising a recombinant construct, for inducing transgenic expression of anti-pathogenic polypeptides, in a fish, is also disclosed. BIOTECHNOLOGY - Preferred Expression Cassette: In (b) of (II), the cecropin polypeptide is a cecropin B polypeptide or cecropin P1 polypeptide. The mature form of the cecropin B polypeptide has an amino acid sequence of Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg -Asn-Ile-Arg-Asn-Gl y-Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile -Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu-Gly. The anti-pathogenic polypeptide is encoded by a nucleotide sequence chosen from fully defined sequences of 189, 186, 123, 120, 111, 108, 174 and 180 base pairs as given in the specification, or a nucleotide sequence having at least 70% sequence identity with the above-mentioned sequences. The mature form of cecropin P1 polypeptide has an amino acid sequence of Ser-Trp-Leu-Ser-Lys-Thr-Ala-Lys-Lys-Leu-G lu-Asn-Ser -Ala-Lys-Lys-Arg-Ile-Ser-Glu-Gly-Ile-Ala-Ile-Ala-Ile -Gln-Gl y-Gly-Pro-Arg. The anti-pathogenic polypeptide is encoded by a nucleotide sequence having a fully defined sequence of 96 or 150 base pairs as given in the specification or a nucleotide sequence having at least 70% sequence identity with above-mentioned sequences. The fusion polypeptide comprising catfish Ig Vh leader and proform of cecropin polypeptide, comprises a fully defined sequence of 58 amino acids as given in the specification. The fusion polypeptide comprising catfish Ig Vh leader and mature form of cecropin polypeptide, has a fully defined sequence of 59 or 49 amino acids as given in the specification. USE - (II) is useful for expressing a polypeptide of interest within a host fish cell, which involves introducing (II) into host fish cell, where the polypeptide of interest is an anti-pathogenic polypeptide and the host fish cell is a cell of a catfish. (II) is useful for enhancing disease resistance in a catfish, which involves introducing (II) into a catfish egg, an culturing the catfish egg under conditions suitable for the maturation and development of the catfish egg into a catfish such as channel catfish, blue catfish or channel-blue hybrid catfish (claimed). (I) is useful for driving expression of a disease resistance genes in a fish such as catfish. (I) is useful for driving constitutive, non-tissue specific expression of anti-pathogenic gene of interest in a fish, and thus the expression of the anti-pathogenic protein in the fish prevents the entry of pathogens such as viruses, parasites and bacteria, into the fish. (I) is useful for enhancing the expression of fish growth hormone, fish-growth releasing factor, and winter flounder antifreeze protein. (I) is useful for driving the expression of sequences encoding a protein of interest, either alone or in combination with sequences encoding other proteins or agents to confer a useful property such as color or texture to a fish. (I) is useful for reducing or inhibiting the expression of gonadotropin releasing hormone or %myostatin% in fish, where (I) is linked to an antisense DNA sequence. ADVANTAGE - (I) enables expression of any nucleotide sequence of interest in a fish, while retaining the expression in a non-tissue specific manner. (I) enables expression of an anti-pathogenic gene in a fish, where the protection is conferred without the need of an inducing stimulus, and the anti-pathogenic protein is expressed throughout the body of the fish. EXAMPLE - Transgenic catfish were constructed to demonstrate that cecropin expression confers disease resistance to catfish. Two strains of transgenic fish were bred. First, one strain of fish was engineered to express the mature cecropin B protein having a sequence of Lys-Trp-Lys-Val-Phe-Lys-Lys-Ile-Glu-Lys-Met-Gly-Arg-Asn-Ile-Arg-Asn-Gly -Ile-Val-Lys-Ala-Gly-Pro-Ala-Ile-Ala-Val-Leu-Gly-Glu-Ala-Lys-Ala-Leu-Gl y, and a second strain of catfish was engineered to express preprocecropin B having a fully defined sequence of 62 amino acids as given in the specification. Initially, a series of constructs were designed that expressed mature cecropin B preprocecropin B or mature cecropin P1 having sequence such as Ser-Trp-Leu-Ser-Lys-Thr-Ala-Lys-Lys -Leu-Glu-Asn -Ser-Ala-Lys-Lys-Arg-Ile-Ser-Glu-Gly-Ile-Ala -Ile-Ala-Ile-Gln-Gly-Gly-Pro-Arg in fish cells under regulatory control of the cytomegalovirus (CMV) enhancer. The CMV enhancer was operably linked to the nucleotide sequences encoding the cecropins of interest. The CMV enhancer was chosen as it was found to promote strong, non-tissue specific expression of transgene in fish cells. The constructs under the control of the enhancer express well in cultured catfish T and B cell lines. For cecropin B constructs, it was necessary to first target the expressed cecropin peptide to the secretory pathway, and second, induce N-terminal processing of the cecropin peptide in the endoplasmic reticulum. Cecropins were initially targeted to the endoplasmic reticulum as preprocecropins, and then, following cleavage of the leader peptide to generate procecropin, four amino-terminal residues were removed by dipeptidyl peptidase to yield the mature cecropin. The first construct (CMV::preprocecropin B) was designed to express the native preprocecropin B peptide. The second construct (CMV::catfish immunoglobulin variable heavy chain leader (Ig Vh)/procecropin B) was designed to express a fusion polypeptide between the catfish Ig Vh and procecropin B having a fully defined sequence of 58 amino acids as given in the specification. The third construct (CMV::catfish Ig Vh leader/arbitrary sequence/cecropin B) was designed to express a fusion polypeptide between the catfish Ig Vh leader and the mature cecropin B having a fully defined sequence of 59 amino acids as given in the specification, with an intervening arbitrary amino acid sequence determined by the cloning strategy. The fourth construct (CMV::catfish Ig Vh leader/cecropin P1) was designed to express a fusion polypeptide between the catfish Ig Vh leader sequence and cecropin P1 which has a fully defined sequence of 49 amino acids as given in the specification. Each construct was cloned between a 5' HindIII and 3' XbaI restriction site. Thus, CMV containing constructs were prepared. (65 pages)

2/7/78 (Item 12 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0342038 DBR Accession No.: 2004-14330 PATENT
New %antibody% that binds GDF-8 or BMP-11, useful in preparing a composition for repairing damaged muscle, increasing muscle mass or strength or increasing glucose tolerance in a mammal - %antibody% production via plasmid expression in host cell for use in disease therapy
AUTHOR: VELDMAN G M; DAVIES M V; SONG K; WOLFMAN N M; GROVE-BRIDGES K; FIELD A; RUSSELL C; VALGE-ARCHER V
PATENT ASSIGNEE: WYETH; CAMBRIDGE ANTIBODY TECHNOLOGY 2004
PATENT NUMBER: WO 200437861 PATENT DATE: 20040506 WPI ACCESSION NO.: 2004-365497 (200434)
PRIORITY APPLIC. NO.: US 419964 APPLIC. DATE: 20021022
NATIONAL APPLIC. NO.: WO 2003IB4748 APPLIC. DATE: 20031022
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A new isolated %antibody% comprises a sequence having 5-258 amino acids and is capable of specifically binding GDF-8 or BMP-11. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a pharmaceutical composition comprising the %antibody%; (2) a method of treating a disorder; (3) an isolated nucleic acid encoding the %antibody%; (4) an expression vector comprising the nucleic acid; (5) a host cell comprising the vector; (6) a method of making an %antibody% that specifically reacts with GDF-8; (7) a method for identifying inhibitors of GDF-8; (8) a method of increasing muscle strength or mass; and (9) an isolated %antibody%. BIOTECHNOLOGY - Preferred %Antibody%: The %antibody% is an scFv fragment expressed by Escherichia coli having ATCC Deposit Designation No. PTA-4741, PTA-4740 or PTA-4739. It binds to a protein comprising

6-amino acid sequence, where the second amino acid from the N-terminus is methionine, the third amino acid from the N-terminus is serine and the fifth amino acid from the N-terminus is isoleucine. It is human. It comprises IgG1 or IgG4. It is capable of inhibiting binding of GDF-8 to ActRIIB. The amino acid sequence of the %antibody% is modified to reduce or alter effector function. The amino acid sequence is modified at residues corresponding to amino acid 117 or 120 of the 330-amino acid sequence. Preferred Host Cell: The host cell is Escherichia coli having ATCC Deposit Designation No. PTA-4741, PTA-4740 or PTA-4739. Preferred Nucleic Acid: The nucleic acid comprises a sequence having 315-786 bp. Preferred Method: Treating a disorder comprises administering the pharmaceutical composition. The pharmaceutical composition is administered for treating or preventing muscle, neuromuscular or bone degenerative disorder. The disorder comprises muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome, congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome, amyotrophic lateral sclerosis, obesity, adipose tissue disorder, syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, type 2 diabetes or damaged myocardial or diaphragm muscle. Making an %antibody% that specifically reacts with GDF-8 comprises: (1) providing a starting repertoire of nucleic acids encoding a variable domain that either includes a CDR3 to be replaced or lacks a CDR3 encoding region; (2) combining the repertoire with a donor nucleic acid encoding the amino acid sequence; (3) expressing the nucleic acids of the product repertoire; (4) selecting a specific antigen-binding fragment specific for GDF-8; and (5) recovering the specific antigen-binding fragment or nucleic acid encoding the binding fragment. Identifying inhibitors of GDF-8 comprises: (1) preparing a first binding mixture comprising the %antibody% and GDF-8; (2) measuring the amount of binding between the %antibody% and GDF-8 in the first mixture; (3) preparing a second binding mixture comprising the %antibody%, GDF-8, a test compound; and (4) measuring the amount of binding between the %antibody% and GDF-8 in the second mixture. Increasing muscle strength or mass comprises administering the %antibody% to a mammal. The method further comprises fusing the nucleic acid encoding the svFv of Myo29, Myo28 or Myo22 with nucleic acids encoding the Fc portion of an immunoglobulin and expressing the fused nucleic acid in a cell. The method also comprises germlining. ACTIVITY - Neuroprotective; Muscular-Gen; Respiratory-Gen; Antidiabetic; Osteopathic; Anorectic. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The %antibody% is useful in preparing a composition for repairing damaged muscle, increasing muscle mass or strength or increasing glucose tolerance in a mammal or for treating or preventing neuromuscular disorder, bone degenerative disorder, muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome, congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome, amyotrophic lateral sclerosis, obesity, adipose tissue disorder, syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, type 2 diabetes or damaged myocardial or diaphragm muscle (claimed). ADMINISTRATION - Dosage comprises 1mug to 150mg, preferably 500mug to 1mg per kg body weight (claimed). The composition is administered via oral or parenteral route. EXAMPLE - No relevant examples given. (117 pages)

2/7/79 (Item 13 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0339652 DBR Accession No.: 2004-11944 PATENT
New agent that modulates metalloprotease-mediated activation of latent %myostatin% comprising a peptide having a peptide portion of a %myostatin% polypeptide, useful for treating metabolic disorders, e.g., diabetes - protein modulation and fusion protein for use in disease therapy and gene therapy
AUTHOR: WOLFMAN N; TOMKINSON K
PATENT ASSIGNEE: WYETH 2004
PATENT NUMBER: WO 200424092 PATENT DATE: 20040325 WPI ACCESSION NO.: 2004-269880 (200425)
PRIORITY APPLIC. NO.: US 486863 APPLIC. DATE: 20030710
NATIONAL APPLIC. NO.: WO 2003US28907 APPLIC. DATE: 20030916
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A new agent that modulates metalloprotease-mediated activation of latent %myostatin% comprises a peptide comprising a peptide portion of a %myostatin% polypeptide, or its derivative comprising a peptide having a mutation of a cleavage site for the metalloprotease. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a method of increasing muscle mass in a subject; and (2) a method of treating a metabolic disorder in a subject. BIOTECHNOLOGY - Preferred Agent: The agent reduces or inhibits or increases metalloprotease-mediated activation of latent %myostatin%. The agent is operatively linked to a second molecule. The second molecule comprises a detectable label and a heterologous polypeptide. The heterologous polypeptide stabilizes the peptide and comprises an Fc domain of an %antibody%. The agent comprises a fusion protein comprising a peptide having an amino acid sequence comprising: (1) KDVIRQLLPKAPPLRELIDQYDVQRADSSDGSLEDDDYHATTETIITMPT; (2) QLLPKAPPLRE
LIDQYDVQRADSSDGSLEDDDYHATTETI; (3) APPLRELIDQYDVQRADSSDGSLEDDDYH. (4)
ELIDQYDVQRADSSDGSLED; or (5) YDVQRADSSD. The fusion protein comprises an operatively linked Fc domain of an %antibody% molecule. The metalloprotease is a bone morphogenetic protein- 1/tolloid (BMP-1/TLD) family member. The BMP-1 /TLD family member is BMP-1, TLD, tolloid-like protein-1 (TLL-I) or tolloid-like protein 2 (TLL-2). The BMP-1 /TLD family member is BMP-1, mammalian TLD (mTLD), mammalian TLL-I (mTLL-1) or mammalian TLL-2 (m-TLL-2). Preferred Method: Increasing muscle mass in a subject comprises administering the agent. Treating a metabolic disorder in a subject comprises administering the agent. The metabolic disorder is a muscle wasting disorder associated with muscular dystrophy, including Duchenne muscular dystrophy; cachexia, including cachexia associated with cancer or acquired immune deficiency syndrome; or sarcopenia, including age-related sarcopenia. The metabolic disorder is diabetes. The metabolic disorder is associated with obesity. ACTIVITY - Antidiabetic; Anorectic; Muscular-Gen; Immunomodulator. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The agent is useful for preparing a composition for treating metabolic disorders, e.g., diabetes, metabolic disorder associated with obesity or muscle wasting disorder associated with muscular dystrophy, including Duchenne muscular dystrophy; cachexia, including cachexia associated with cancer or acquired immune deficiency syndrome; or sarcopenia, including age-related sarcopenia. ADMINISTRATION - The composition is administered via oral or parenteral route. No dosage given. (95 pages)

2/7/80 (Item 14 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0323883 DBR Accession No.: 2003-25023 PATENT
Use of agents that modulate PGC-1alpha expression or activity, for modulating type I muscle formation or treating a disorder associated with aberrant type I muscle formation e.g. heart failure, diabetes or a mitochondrial myopathy - adeno virus or adeno-associated virus vector-mediated gene transfer and expression in host cell, transgenic animal and %antibody% for gene therapy and drug screening
AUTHOR: SPIEGELMAN B M; LIN J
PATENT ASSIGNEE: DANA FARBER CANCER INST INC 2003
PATENT NUMBER: WO 200368944 PATENT DATE: 20030821 WPI ACCESSION NO.: 2003-689670 (200365)
PRIORITY APPLIC. NO.: US 357069 APPLIC. DATE: 20020213
NATIONAL APPLIC. NO.: WO 2003US4792 APPLIC. DATE: 20030213
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Modulating type I muscle formation comprising contacting a cell with an agent that modulates PGC-1alpha expression or activity, so that type I muscle formation is modulated, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method for identifying a compound capable of modulating type

l muscle formation comprising contacting a cell with a compound, and determining whether PGC-1alpha expression or activity is modulated; (2) a method for identifying a compound capable of treating a disorder associated with aberrant type l muscle formation comprising assaying the ability of the compound to modulate the expression or activity of PGC-1alpha; (3) compounds identified by the method of (1) or (2); (4) a method for treating a subject having a disorder associated with aberrant type l muscle formation comprising administering to the subject an agent capable of modulating PGC-1alpha expression or activity; (5) a method for increasing type l muscle formation in a subject comprising administering to the subject an agent capable of increasing PGC-1alpha expression or activity; and (6) a non-human transgenic animal comprising an exogenous PGC-1alpha nucleic acid molecule, where the exogenous PGC-1alpha nucleic acid molecule is expressed in the skeletal muscle of the animal. WIDER DISCLOSURE - Expression vectors and host cells comprising the PGC1alpha nucleic acids, and %antibodies% that specifically bind PGC-1alpha polypeptides, are also disclosed. BIOTECHNOLOGY - In modulating type l muscle formation, PGC-1alpha expression or activity is increased or decreased. Preferably, type l muscle formation is increased. The agent used in any of the methods cited above is a PGC-1alpha nucleic acid molecule, a PGC-1alpha polypeptide, or a small molecule. The PGC-1alpha nucleic acid molecule is derived from a human, and comprises a fully defined sequence of 3023 bp given in the specification. The PGC-1alpha nucleic acid molecule is contained within a vector, where the vector is an adenoviral or an adeno-associated vector. The PGC-1alpha polypeptide is derived from a human, and comprises a fully defined sequence of 798 amino acids given in the specification. The cell is a muscle cell, preferably a skeletal muscle cell consisting of a type l muscle cell or a type II muscle cell. The method is performed in vitro or in vivo, preferably in a mouse or in a human. In identifying a compound capable of modulating type l muscle formation or treating a disorder associated with aberrant type l muscle formation, PGC-1alpha expression or activity is increased. PGC-1alpha expression is measured by Northern blotting. Determining whether PGC-1alpha expression or activity is modulated comprises determining whether expression of at least one of myoglobin, troponin I slow, troponin I fast, MCAD, COX II, COX IV, or cytochrome c is modulated. Preferred Transgenic Animal: The animal is preferably a mouse. The exogenous PGC-1alpha nucleic acid molecule is operatively linked to a muscle specific promoter. The muscle specific promoter is the muscle creatine kinase promoter, the dystrophin promoter, the %myostatin% promoter, the GDF-8 promoter, the UCP-3 promoter, the MyoD promoter, the MEF2 promoter, the myosin heavy chain promoter, the myosin light chain promoter, or a troponin promoter. Expression of at least one of myoglobin, troponin I slow, MCAD, Cox II, COX IV, or cytochrome c is upregulated in the muscle cells of the animal. ACTIVITY - Cardiant; Antidiabetic; Anorectic; Immunomodulator; Antidepressant. No biological data given. MECHANISM OF ACTION - Gene Therapy. USE - The agents that modulate PGC-1alpha expression or activity are useful for modulating type l muscle formation or treating a disorder associated with aberrant type l muscle formation, e.g. heart failure, disuse atrophy, a mitochondrial myopathy, or a systemic metabolic disorder such as diabetes, obesity, cachexia or anorexia (all claimed). The PGC-1alpha nucleic acid molecules, polypeptides, %antibodies% and modulators are useful in drug screening assays or in gene therapy. The transgenic animals are useful in screening assays designed to identify agents or compounds that are involved with type l muscle formation. ADMINISTRATION - Administration may be intravenous, intradermal, subcutaneous, oral (i.e. inhalation), transdermal (topical), transmucosal, or rectal. No dosage given. EXAMPLE - No relevant example given.(114 pages)

2/7/81 (Item 15 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0319713 DBR Accession No.: 2003-20853 PATENT
New flow electroporation device for encapsulating allosteric effectors (e.g. inositol hexaphosphate) of hemoglobin in cells (e.g. erythrocytes) comprises walls defining a flow channel, an inlet flow portal and an outlet flow portal - flow electroporation for cell transfection with application in gene therapy of heart condition, anemia and lung disorder
AUTHOR: DZEKUNOV S M; LEE H J; LI L; SINGH V; LIU L; HOLADAY J W
PATENT ASSIGNEE: DZEKUNOV S M; LEE H J; LI L; SINGH V; LIU L; HOLADAY J W 2003
PATENT NUMBER: US 20030059945 PATENT DATE: 20030327 WPI ACCESSION NO.: 2003-567141 (200353)
PRIORITY APPLIC. NO.: US 80272 APPLIC. DATE: 20020221
NATIONAL APPLIC. NO.: US 80272 APPLIC. DATE: 20020221
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A flow electroporation device (I), is new. DETAILED DESCRIPTION - A flow electroporation device (I) comprises: (a) walls defining a flow channel configured to receive and to transiently contain a continuous flow of a suspension comprising particles;(b) an inlet flow portal in fluid communication with the flow channel, where the suspension can be introduced into the flow channel through the inlet flow portal; and (c) an outlet flow portal in fluid communication with the flow channel, where the suspension can be withdrawn from the flow channel through the outlet flow portal. The walls defining the flow channel comprises a first electrode plate forming a first wall of the flow channel and a second electrode plate forming a second wall of the flow channel opposite the first wall, where the area of the electrodes contact with the suspension, and the distance between the electrodes is chosen so that the thermal resistance of the flow channel is less than 4 degreesC per Watt. The paired electrodes are placed in electrical communication with a source of electrical energy, where an electrical field is formed between the electrodes. The suspension of the particles flowing through the flow channel can be subjected to an electrical field formed between the electrodes. INDEPENDENT CLAIMS are included for the following: (1) transfecting a cell, comprising providing an expression vector coding for a desired protein or peptide and introducing the vector into the cell by flow electroporation; (2) delivering a therapeutic agent to a patient, comprising incorporating the therapeutic agent into platelets by electroporation, and administering the platelets to the patient; and (3) treating a patient with a therapeutic protein, comprising transfecting a cell population with an expression vector that codes for the desired protein by flow electroporation. BIOTECHNOLOGY - Preferred Device: The electrode plates in the device further comprises a gasket formed from an electrically non-conductive material and disposed between the first and second electrode plates to maintain the electrode plates in spaced-apart relation, the gasket defining a channel forming opposed side walls of the flow channel. The gasket forms a seal with each of the first and second electrode plates. The device comprises flow channels and the gasket comprises channels forming opposed sidewalls of each of the channels. One of the inlet and outlet flow portals comprises a bore formed in one of the electrode plates and in fluid communication with the flow channel. In addition, the device comprises a cooling element operatively associated with the flow channel to dissipate heat. The cooling element comprises a thermoelectric cooling element, a cooling fluid flowing in contact with the electrode, or a heat sink operatively associated with the electrode. The resistance of the flow channel is 0.5-4, preferably 1.5-2.5 degreesC per watt. The first electrode comprises an elongated, electrically conductive structure, and the second electrode comprises a tubular, electrically conductive structure. The electrodes are concentrically arranged so that the second, tubular electrode surrounds the first electrode in spaced-apart relation, and the flow channel is disposed within an annular space defined between the first and second electrodes. The electrodes form at least a portion of the walls defining the flow channel. The device may also comprise concentric annular spacers for maintaining the first and second electrodes in spaced-apart, concentric relation. The device is arranged in series or in parallel with a second, like device. Preferred Method: In transfecting a cell, 50-95, preferably 70-80 % of the cells transfected by electroporation express the desired protein or are viable. The desired protein is b-cell differentiation factor, b-cell growth factor, mitogenic cytokine, chemotactic cytokine, colony stimulating factor,

angiogenesis factor, cadherin, selectin, integrin, NCAM, ICAM, L1, t-cell replacing factors, differentiation factor, transcription factor, mRNA, heat shock protein, nuclear protein complex, RNA/DNA oligomer, interferon (IFN)-alpha, IFN-beta, IFN-omega, interleukin (IL)1 to IL18, leptin, %myostatin% macrophage stimulating protein, platelet-derived growth factor, tumor necrosis factor (TNF)-alpha, TNF-beta, NGF, CD40L, CD137L/4-1BBL, human lymphotoxin-beta, TNF-related apoptosis-inducing ligand, monoclonal %antibody%, fragments of monoclonal %antibody%, granulocyte-colony stimulating factor (G-CSF), macrophage (M)-CSF, granulocyte monocyte (GM)-CSF, platelet derived growth factor (PDGF), IL1-alpha, IL-beta, fibroblast growth factor (FGF), IFN-gamma, IP-10, PF4, GRO, 9E3, erythropoietin, endostatin, angiostatin, vascular endothelial growth factor (VEGF), or soluble receptor and any of their fragments or combinations. Preferably, the desired proteins are erythropoietin, endostatin, angiostatin, IL12, IL2, or their fragments. In delivering a therapeutic agent to a patient, the electroporation is flow electroporation. The therapeutic agent is AGM-1470 (TNP-470); MetAP-2; growth factor antagonists; %antibodies% to growth factors; growth factor receptor antagonists; TIMP; batimastat; marimastat; genistein SU5416; alphaVbeta3/5; retinoic acid; fenretinide; 1alphaepihydrocortisol, corteloxone, tetrahydrocortisone and 17alphahydroxyprogesterone; staurosporine, MDL 27032; vitamin D derivatives including 22-oxa-1 alpha, and 25-dihydroxyvitamin D3; arachidonic acid inhibitors including indomethacin and sulindac; tetracycline derivatives including minocycline; thalidomide derivatives; 2-methoxyestradiol; tumor necrosis factor-alpha; interferon-gamma-inducible protein 10 (IP-10); IL1 and IL12; IFN alpha, beta or gamma; angiostatin protein or plasminogen fragments; endostatin protein or collagen 18 fragments; proliferin-related protein; group B streptococcus toxin; CM101; CAI; troponin I; squalamine; nitric oxide synthase inhibitors including L-NAME; thrombospondin; wortmannin; amiloride; spironolactone; ursodeoxycholic acid; bufalin; suramin; tecogalan sodium; linoleic acid; captopril; irsogladine; FR-118487; triterpene acids; castanospermine; leukemia inhibitory factor; lavendustin A; platelet factor-4; herbimycin A; diaminoantraquinone; taxol; aurin-tricarboxylic acid; DS-4152; pentosan polysulphite; radicicol; fragments of human prolactin; erbstatin; eponemycin; shark cartilage; protamine; Louisianin A, C and D; PAF antagonist WEB 2086; auranofin; ascorbic ethers; or sulfated polysaccharide D 4152. ACTIVITY - Cardiant; Vasotropic; Antiarteriosclerotic; Cerebroprotective; Antianemic. No biological data is given. MECHANISM OF ACTION - Cell therapy. USE - The device and methods are useful for the encapsulation of biologically active substances in various cell populations (e.g. erythrocytes) in blood by electroporation to achieve therapeutically desirable changes in the physical characteristics of the cell populations in the blood. The cells may be used for treating individuals who are experiencing lowered oxygenation of their tissues due to the inadequate function of their lungs or circulatory system, which may be due to cardiovascular or hematological diseases, such as heart failure, ischemia, myocardial infarction, stroke, anemia, arteriosclerosis, or blood loss. The cells may also be used to accelerate detoxification processes by improving oxygen supply, to improve the efficacy of various cancer treatments, and to enhance the athletic performance of humans or animals. ADMINISTRATION - Treating a human or animal for any of the above diseases is done by transfusing 0.5-6 units of treated blood product, such as platelets. The platelets are administered to the patient intravenously. (All claimed.) EXAMPLE - No relevant example given.(59 pages)

2/7/82 (Item 16 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0319355 DBR Accession No.: 2003-20495 PATENT
New %myostatin% peptide, useful for preparing a composition for treating a disorder comprising degeneration or wasting of muscle in a vertebrate subject - vector-mediated gene transfer and expression in host cell for recombinant protein production for disease gene therapy and recombinant vaccine

AUTHOR: BARKER C A; MORSEY M
PATENT ASSIGNEE: METAMORPHIX INT INC 2003
PATENT NUMBER: US 20030065137 PATENT DATE: 20030403 WPI ACCESSION NO.: 2003-540801 (200351)
PRIORITY APPLIC. NO.: US 74152 APPLIC. DATE: 20020211
NATIONAL APPLIC. NO.: US 74152 APPLIC. DATE: 20020211
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A %myostatin% peptide comprising a sequence having 3-100 or 3-200 amino acids, is new. It comprises at least one epitope of %myostatin%. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a %myostatin% multimer comprising two or more %myostatin% immunogens, each of which comprises at least 3 amino acids defining at least one epitope of %myostatin%; (2) a %myostatin% immunoconjugate comprising at least one %myostatin% peptide or multimer linked to an immunological carrier; (3) a vaccine composition comprising the %myostatin% peptide, multimer or immunoconjugate and an excipient or adjuvant; (4) eliciting an immune response against a %myostatin% immunogen in a vertebrate subject; (5) treating a disorder comprising degeneration or wasting of muscle in a vertebrate subject; (6) modulating GDF11 activity in a vertebrate subject; (7) a polynucleotide encoding a %myostatin% peptide, multimer or immunoconjugate; (8) a recombinant vector comprising the polynucleotide of (7) and control elements operably linked to the polynucleotide; (9) a host cell transformed with the recombinant vector of (8); (10) producing a recombinant %myostatin% peptide, multimer or immunoconjugate; and (11) an isolated %antibody% reactive with the %myostatin% peptide. BIOTECHNOLOGY - Preferred Peptide: The %myostatin% peptide comprises a sequence having 3-30 or 3-15 amino acids. It is derived from the region of %myostatin% spanning amino acids 45-376 or 235-376 of the 376-amino acid sequence or amino acids 1-275, 25-300, 50-325 or 75-350 of the 350-amino acid sequence. Preferred Multimer: The %myostatin% multimer comprises a molecule of the formula (MP-X-MP)y and (MP)n. X = peptide linkage, amino acid spacer group or leukotoxin peptide; n = greater than or equal to 1; and y = greater than or equal to 1. Preferred Carrier: The immunological carrier is leukotoxin peptide. Preferred Method: Eliciting an immune response against a %myostatin% immunogen in a vertebrate subject comprises administering the vaccine composition. The immune response elicited reduces endogenous %myostatin% activity in the vertebrate subject and results reduction in body fat content, or an increase in body weight, muscle mass, number or size of muscle cells, muscle strength, mammary gland tissue, lactation, appetite or food uptake or the life span of the vertebrate subject. Treating a disorder comprising degeneration or wasting of muscle in a vertebrate subject comprises administering the vaccine composition or polynucleotide. Modulating GDF11 activity in a vertebrate subject comprises administering the vaccine composition. Producing a recombinant %myostatin% peptide, multimer or immunoconjugate comprises providing a population of host cells and culturing the population of cells for expression of the %myostatin% peptide, multimer or immunoconjugate. ACTIVITY - Vulnerary. No biological data is given. MECHANISM OF ACTION - Gene therapy. USE - The %myostatin% peptide is useful for preparing a composition for treating a disorder comprising degeneration or wasting of muscle in a vertebrate subject (claimed). ADMINISTRATION - Dosage comprises 1 micro-g-1 mg, preferably 5-200 micro-g/kg. The composition is administered via parenteral route. EXAMPLE - No relevant examples given. (42 pages)

2/7/83 (Item 17 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0316220 DBR Accession No.: 2003-17360 PATENT
New composition comprising an isolated stromal cell capable of supporting the in vitro proliferation and maintenance of stem cells in combination with a stem cell, useful for supporting embryonic and adult stem cells - genetically modified cell culture, growth factor and genetic material for use in tissue engineering
AUTHOR: LUFT C; WILKISON W O; CHEATHAM B; GIMBLE J M; HALVORSEN Y C
PATENT ASSIGNEE: ARTECEL SCI INC 2003

PATENT NUMBER: WO 200340346 PATENT DATE: 20030515 WPI ACCESSION NO.: 2003-441563 (200341)
PRIORITY APPLIC. NO.: US 344555 APPLIC. DATE: 20011109
NATIONAL APPLIC. NO.: WO 2002US36317 APPLIC. DATE: 20021112
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A composition comprising an isolated stromal cell capable of supporting the in vitro proliferation and maintenance of stem cells in combination with a stem cell, is new. DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for a method for the growth and maintenance of cultured stem cells by isolating tissue-derived stromal cells, and culturing the stromal cells in culture media with stem cells. BIOTECHNOLOGY - Preferred Composition: The composition comprises human stromal cell. An exogenous genetic material has been introduced into the stromal cell. The stromal cell secretes a protein. The protein secreted is a growth factor, cytokine or any protein promoting the proliferation of the stem cell. The stromal cell is irradiated. The stromal cells is derived from adipose tissue, bone marrow, ligamentous tissue or tendon, skeletal muscle, smooth muscle, bone, cartilage, connective tissue, peripheral blood, umbilical cord blood, or placenta. The stem cell can be embryonic or adult in origin. The stem cell expresses telomerase. The stem cell is selected from neuronal, liver, hematopoietic, umbilical cord blood, epidermal, gastrointestinal, endothelial, muscle, mesenchymal and pancreatic stem cell. The stem cell remains undifferentiated. Preferred Method: The method for growth and maintenance of cultured stem cells further comprises culture supplemented with growth factors, cytokines or chemokines, which are selected from leukemia inhibitory factor, interleukin 1 (IL-1) through IL-13, IL-15 through IL-17, IL-19 through IL-22, granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage-colony stimulating factor (M-CSF), erythropoietin (Epo), thrombopoietin (Tpo), Flt3-ligand, B cell activating factor, artemin, bone morphogenic protein factors, epidermal growth factor (EGF), glial derived neurotrophic factor, lymphotactin, macrophage inflammatory proteins, %myostatin% , neurturin, nerve growth factors, platelet derived growth factors, placental growth factor, pleiotrophin, stem cell factor, stem cell growth factors, transforming growth factors, tumor necrosis factors, vascular endothelial cell growth factors, fibroblast growth factors, and FGF-acidic and basic fibroblast growth factor. The isolated stromal cells are genetically engineered to express a growth factor, cytokine or chemokine. The growth factors, cytokines and chemokines can promote or inhibit the differentiation of the stem cells. The isolated stromal cells are irradiated prior to culturing with the stem cells. The stem cells are maintained in a differentiated or undifferentiated state. USE - The composition and stromal cells are useful for supporting embryonic and adult stem cells, and for the growth and maintenance of cultured stem cells. They are also useful as feeder layers in the isolation, culture and maintenance of adult, embryonic and other stem cells. EXAMPLE - The ability of human adipose-derived stromal cells to support the proliferation and differentiation of human umbilical cord blood CD34+ hematopoietic progenitor cells in vitro was examined. Confluent cultures of adipose-derived stromal cells were established in 24 well plates (6 x 10 (to the power of 4) cells per well). Umbilical cord blood specimens were depleted of contaminating erythrocytes by treatment with hetastarch and of contaminating granulocytes by Ficoll density centrifugation. The remaining UCB mononuclear cells were lineage depleted according to the StemSep (TM) (StemCells, Vancouver, BQ protocol; this relies on immunomagnetic negative cell selection using a cocktail of %antibodies% directed against CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, and glycophorin A. In the last purification step, the lin- UCB cells were stained with CD34 %antibodies% and sorted by flow cytometry. Up to 10000 of the final CD34+ UCB cells have been cocultured in individual wells with a confluent adipose-derived stromal cell layer. Cultures were maintained in the absence of exogenous cytokines for periods of 12-days, 3 weeks, or 6 weeks. At the end of these periods, individual wells were harvested by trypsin/EDTA digestion and analyzed by flow cytometry using a combination of the following %antibody% combinations (fluorescent tags indicated in parentheses): CD45 (FITC), CD34 (APC), and either CD7, CD10, or CD38 (PE). The results show that hematopoietic cells from 12 day adipose stroma co-cultures were examined for total cell expansion, CD34+ cell expansion or seeded on MS5 cells for 5 weeks and the expansion of myeloid long term culture initiating (LTC) cells. In the absence of exogenous cytokines, adipose-derived stromal cells supported a 5.1 fold expansion of total hematopoietic cell numbers (average, n = 4 stromal donors, n = 2 UCB donors; range 2 - 9.4). This corresponded to a 2 fold expansion of the CD34+ UCB cell population (average, n = 4 stromal donors, n = 2 UCB donors; range 1.4 - 3.3).(33 pages)

2/7/84 (Item 18 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0315095 DBR Accession No.: 2003-16235 PATENT
Identifying mammalian cell capable of producing a proteinaceous molecule, by analyzing post-translational modification on a protein produced by mammalian cell, and determining whether protein comprises the modification - recombinant protein production via cell culture transfection for use in disease therapy
AUTHOR: OPSTELTEN D J E; KAPTEYN J C; PASSIER P C J J; BRUS R H P; BOUT A
PATENT ASSIGNEE: CRUCELL HOLLAND BV 2003
PATENT NUMBER: WO 200338100 PATENT DATE: 20030508 WPI ACCESSION NO.: 2003-421522 (200339)
PRIORITY APPLIC. NO.: WO 2002257 APPLIC. DATE: 20020419
NATIONAL APPLIC. NO.: WO 2002NL686 APPLIC. DATE: 20021029
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Identifying (M1) a mammalian cell capable of producing a proteinaceous molecule having a predetermined post-translational modification, comprising analyzing the post-translational modification on a protein produced by the mammalian cell, and determining whether the protein comprises the predetermined post-translational modification, is new. DETAILED DESCRIPTION - Identifying (M1) a mammalian cell capable of producing a proteinaceous molecule having a predetermined post-translational modification, comprising: (a) analyzing the post- translational modification on a protein produced by the mammalian cell; and (b) determining whether the protein comprises the predetermined post-translational modification. The mammalian cell is selected which is capable of producing a proteinaceous molecule, by analyzing the presence or absence of a tissue specific marker or a combination of tissue specific markers in the mammalian cell or on the cell surface of the mammalian cell, which marker or combination of the markers is indicative for the predetermined post-translational modification to be present on the proteinaceous molecule, and selecting the mammalian cell on the basis of the presence or absence of the tissue specific markers. The mammalian cell is obtained from a heterogeneous cell population, the mammalian cell being capable of producing a proteinaceous molecule, by sorting cells on the basis of the post-translational modifications on proteins produced by the cells in the heterogeneous cell population, and selecting the cells capable of producing proteins comprising the predetermined post-translational modification. INDEPENDENT CLAIMS are also included for the following: (1) identifying, and/or selecting, and/or obtaining a mammalian cell capable of producing a proteinaceous molecule comprising a predetermined post-translational modification of M1, where the predetermined post-translational modifications are present on a proteinaceous molecule that is recombinantly expressed in the mammalian cell; (2) a pharmaceutical composition (I) comprising recombinantly produced erythropoietin having a predetermined post-translational modification, where the recombinantly produced erythropoietin is obtained using M1, and has a lower erythropoietic effect as compared to erythropoietin not having the predetermined post-translational modification, and a carrier; (3) recombinantly produced erythropoietin (II) comprising at least one post-translational modification selected from sialyl Lewis x structure, Lewis x structure, alpha,3- linked fucose attached to N-acetyl-glucosamine, LacidNAc structure, terminal N-acetyl-glucosamine group and a terminal galactose group; (4) use of a mammalian cell obtainable by M1 for the production

of a proteinaceous molecule comprising a predetermined post-translational modification; (5) a pharmaceutical preparation comprising erythropoietin-like molecules selected from erythropoietin, one or more muteins of erythropoietin, one or more derivatives of erythropoietin, and a composition of one or more fractions of erythropoietin or erythropoietin-like molecules sialylated to a varying degree; (6) preventative and/or therapeutic treatment of a disorder chosen from ischemia, a reperfusion injury, a hypoxia-induced disorder, an inflammatory disease, neurodegenerative disorder, and acute damage to the central-or peripheral nervous system, comprising administering to a human or animal subject, a protein content basis a lower erythropoietic activity in vivo than epoetin alfa, and/or the presence of erythropoietin-like molecules that once administered parenterally to a human or an animal subject are cleared from the bloodstream at a faster rate than epoetin alfa; (7) producing in a mammalian cell proteinaceous molecules in need of a glycosylation structure chosen from (sialyl) Lewis X and/or LacdiNac containing N-linked glycan structures, characterized in that the cell expresses nucleic acid encoding E1A from an adenovirus, with the proviso that when the proteinaceous molecule is erythropoietin the mammalian cell is not a PER.C6 (RTM) cell, when the proteinaceous molecule is protein C the mammalian cell is not a HEK293 cell or a Syrian hamster AV12-664 cell, when the proteinaceous molecule is glycodelin or tissue factor pathway inhibitor the mammalian cell is not a HEK293 cell, and when the proteinaceous molecule is matrix metalloprotease 1 the mammalian cell is not a HT1080 cell; (8) producing a fraction enriched in a proteinaceous molecule having N-linked glycans comprising (sialyl)Lewis X and/or LacdiNac structures, by recombinantly expressing the proteinaceous molecule in a cell that expresses nucleic acid encoding E1A from an adenovirus, and fractionating the proteinaceous molecules so produced, thus obtaining a fraction which is enriched in molecules having the N-linked glycans comprising (sialyl)Lewis X and/or LacidNac structures; (9) fractionating a mixture comprising proteinaceous molecules that comprise Lewis X structures, the method employing binding of the molecules to an AAL lectin; (10) a composition comprising erythropoietin-like molecules chosen from erythropoietin, one or more muteins of erythropoietin, and one or more derivatives of erythropoietin, characterized in that the average number of lewis-X structures on N-linked glycans per erythropoietin-like molecule is at least 2.2; and (11) a composition of erythropoietin-like molecules is characterized in that it is recombinantly producible in a mammalian cell comprising nucleic acid encoding E1A from an adenovirus. BIOTECHNOLOGY - Preferred Method: In M1, the predetermined post-translational modification comprises glycosylation which involves one modification selected from Lewis x, sialyl Lewis x, Galnac structure, GlcNac structure, alpha1,3-linked fucose attached to N-acetyl-glucosamine, terminal N-acetyl-glucosamine, terminal galactose, bisecting N-acetyl-glucosamine, sulfate group and sialic acid. The composition of erythropoietin-like molecules is obtainable by recombinant expression of erythropoietin or a mutein of erythropoietin in transfected Chinese Hamster Ovary cells, Baby Hamster Kidney cells, or transfected human cells, Baby Hamster Kidney cells, or transfected human cells followed by one or more purification steps. The erythropoietin-like molecules have been produced by M1. The erythropoietin-like molecules have been produced on a cell comprising the E1A region of an adenovirus. The composition of erythropoietin-like molecules has been obtained by subjecting erythropoietin-like molecules to treatment with an enzyme such as neuraminidase or a chemical substance such as an acid to lower the average number of sialic acid residues per erythropoietin-like molecule. Preferred Protein: The erythropoietic is produced on a mammalian cell obtainable using M1. The erythropoietin-like molecules have been obtained by subjecting erythropoietin-like molecules to treatment to lower the average number of sialic acid residues per erythropoietin-like molecule. The proteinaceous molecules are selected from erythropoietin, transferring, a glycodelin such as glycodelin A (PP14), Nerve Growth Factor (NGF), Brain-derived neurotrophic factor, Neurotrophin-3, - 4/5 and -6, Ciliary neurotrophic factor, Leukemia inhibitory factor, Cardiotrophin-1, Oncostatin- M, an Interleukin, GM-CSF, IGF-1 and -2, TGF-beta, Glial-derived neurotrophic factor, Neurturin, Persephin, %Myostatin% , Fibroblast Growth Factor-1, -2 and -5, Amphiregulin, Acetylcholine receptor inducing activity, Netrin-1 and -2, Neuregulin-2 and -3, Pleiotrophin, Midkine, Stem cell Factor (SCF), Agrin, CSF-1, PDGF, Saposin C, soluble complement receptor-1, alpha-1 acid glycoprotein, acute-phase proteins, E-selecting ligand-1, LAM-1, Carcinoembryonic antigen-like CD66 antigens, peripheral lymph node Addressing, CD75, CD76, CD45RO, CD21, p-selecting glycoprotein ligand-1, GlyCAM-1, Mucin-type glycoproteins, CD34, podocalyxin, alpha1-antichymotrypsin, alpha1-protease inhibitor, alpha- amylase, salivary proline-rich glycoproteins, SERP-1, interferon- beta, beta-trace protein C, Urokinase, Schistosome glycoprotein, alpha-fetoprotein, human pregnancy proteins such as gonadotropic hormones such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), human Choriogonadotropin (hcG), or fragments or variants of any of these that are capable of receiving the N- linked glycan structures. The proteinaceous molecule is erthyropoietin or an erthyropoietin-like molecule. The fraction is enriched by a method comprising a affinity purification step that employs the glycan structures. The purification step employs binding of the molecules to a lectin or to a monoclonal %antibody% that binds to the N-linked glycans comprising (sialyl)Lewis X and/or LacdiNac structures. The purification step employs binding of the N-linked glycans comprising (sialyl)Lewis X and/or LacdiNac structures to an AAL lectin. The N-linked glycans on the erythropoietin-like molecules are mainly biatennary structures. A pharmaceutical preparation comprising molecules from a fraction or composition. ACTIVITY - Vasotropic; Antiinflammatory. No biological data is given. MECHANISM OF ACTION - None given. USE - M1 is useful for identifying, selecting, obtaining a mammalian cell capable of producing a proteinaceous molecule comprising a predetermined post-translational modification, where the mammalian cell is of neural origin, human cell, or immortalized. The mammalian cell has been provided with a nucleic acid encoding the E1 region, or its part, from human adenovirus in such a way that the mammalian cell harbors the nucleic acid in an expressible form. The proteinaceous molecule is erythropoietin. M1 is also useful for producing a proteinaceous molecule and expressing the proteinaceous molecule in the mammalian cell. The mammalian cell is PER.C6 (RTM). The extra step of purifying the proteinaceous molecule from the mammalian cell culture. The purification comprises a step employs the predetermined post- translational modification. The purification comprises a step in which an %antibody% is employed that is specific for an epitope present modification, and comprises a lectin-binding step. (I) is useful for the preparation of a medicament for the treatment of a disorder selected from ischemia, reperfusion injury, hypoxia-induced disorder, inflammatory disease, neurodegenerative disorder, and acute damage to the central-or peripheral nervous system. (All claimed.) EXAMPLE - No relevant example is given. (174 pages)

2/7/85 (Item 19 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0305150 DBR Accession No.: 2003-06935 PATENT
Transgenic non human animal whose genome contains sequence of truncated Activin Type II receptor gene, %myostatin% prodomain or follistatin gene and muscle-specific promoter, and expression of gene increases muscle mass - plasmid vector-mediated gene transfer for transgenic fowl, bird, pig, fish, cattle and mouse construction
AUTHOR: LEE S; MCPHERRON A C
PATENT ASSIGNEE: UNIV JOHNS HOPKINS SCHOOL MEDICINE 2002
PATENT NUMBER: WO 200285306 PATENT DATE: 20021031 WPI ACCESSION NO.: 2003-093057 (200308)
PRIORITY APPLIC. NO.: US 841730 APPLIC. DATE: 20010424
NATIONAL APPLIC. NO.: WO 2002US13103 APPLIC. DATE: 20020424
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A transgenic non human animal (TA) whose genome contains a sequence having truncated Activin Type II receptor gene, %myostatin% prodomain or follistatin gene and muscle-specific promoter linked and integrated into genome of animal,

is new. Expression of sequence results in elevated levels of receptor, %myostatin% prodomain or follistatin and increased muscle mass in animal as compared to nontransgenic animal. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an expression cassette (I) comprising a DNA segment encoding a truncated Activin RIIB receptor gene, %myostatin% prodomain gene or a follistatin gene, operably linked to a muscle-specific control sequence;(2) a cell or cell line isolated from (TA), where the cells express truncated Activin Type II receptor, %myostatin% prodomain, or follistatin, respectively; (3) inhibiting (M) %myostatin% binding to an Activin type II receptor, comprising contacting %myostatin% with follistatin, thus inhibiting binding to the receptor; (4) producing animal food products having increased muscle mass, comprising: (a) introducing a transgene encoding follistatin, %myostatin% propeptide or a truncated Activated Type II receptor into germ cells of a pronuclear embryo of the animal; (b) implanting the embryo into the oviduct of the pseudopregnant female thus allowing the embryo to mature to full term progeny; (c) testing the progeny for presence of the transgene to identify transgene-positive progeny; (d) cross-breeding transgene-positive progeny to obtain further transgene-positive progeny; and (e) processing the progeny to obtain foodstuff; and (5) producing avian, porcine, piscine or bovine food products having increased muscle mass, comprising: (a) introducing a transgene encoding follistatin, %myostatin% propeptide or a truncated Activin Type II receptor into an embryo of an avian, porcine, piscine or bovine animal; (b) culturing the embryo under conditions by which progeny are hatched; (c) testing the progeny for presence of the transgene to identify transgene-positive progeny; (d) cross-breeding transgene-positive progeny; and (e) processing the progeny to obtain foodstuff. WIDER DISCLOSURE - (1) growth differentiation factor (GDF) receptors or their variants; (2) compositions that affect %myostatin% signal transduction in a cell;(3) polynucleotides encoding GDF receptors i.e promyostatin polypeptide and pro-GDF-11 polypeptide, or their mutants; (4) %antibodies% that specifically interacts with GDF receptors, and their use; (5) identifying agents that interacts with GDF receptors; (6) a substantially purified peptide portion of a promyostatin polypeptide and pro-GDF-11 polypeptide; (7) a virtual representation of GDF receptor or a functional peptide portion of GDF receptors; (8) a substantially purified proteolytic fragment of GDF polypeptide (a pro-GDF polypeptide) or a functional peptide portion of it; (9) a computer system which stores or manipulates coordinate information obtained by crystallographic or nuclear magnetic resonance (NMR) analysis, or amino acid or nucleotide sequence information; (10) pharmaceutical compositions containing an agent which is useful for modulating %myostatin% signal transduction in a cell; (11) oligonucleotide portions of a polynucleotide encoding a GDF receptor; and (12) GDF receptor-binding agents that block the binding of GDF to its receptor. BIOTECHNOLOGY - Preferred Animal: The muscle-specific promoter is a myosin light chain promoter/enhancer. The Activin Type II receptor is RIIA or RIIB. The truncated Activin RIIB receptor lacks kinase activity. The truncated Activin RIIB receptor comprises amino acid residues 1-174 of Activin RIIB. The %myostatin% prodomain comprises 1 to 262 of the promyostatin polypeptide having a 375 (S1), 376 (S2), 376 (S3), 374 (S4), 375 (S5), 375 (S6), 375 (S7), 375 (S8), 375 (S9) or 374 (S10) residue amino acid sequence, given in the specification, or a functional peptide portion of the %myostatin% prodomain. The %myostatin% prodomain comprises an amino acid residues 20-263 of (S2), (S3), 20-262 of (S1), (S4)-(S10), or a functional peptide portion of the sequence. The %myostatin% prodomain further comprises a %myostatin% signal peptide. The DNA construct has been introduced into an ancestor of the animal. The DNA construct is introduced to the animal or ancestor of the animal at an embryonic stage. The follistatin protein is truncated, mutant or other variant form of follistatin protein as compared to the wild-type. The DNA construct is in a MDAF2 expression plasmid containing DNA segment encoding a follistatin protein. The animal is a mammal such as mouse, porcine or bovine. The animal is an avian species such as chicken or turkey. The animal is an aquatic species, preferably a fin fish. The finfish is a salmon, trout, char, ayu, carp, crucian carp, goldfish, roach, whitebait, eel, conger eel, sardine, zebrafish, flying fish, sea bass, sea bream, parrot bass, snapper, mackerel, horse mackerel, tuna, bonito, yellowtail, rockfish, fluke, sole, flounder, blow fish or filefish. The aquatic species is a clam, cockle, mussel, periwinkle, scallop, conch, snail, sea cucumber, ark shell, oyster, turban shell, abalone, lobster, prawn; shrimp, crab, squilla, krill, langostino, crayfish/crawfish, Annelida, alligator, turtle, frog or sea urchins. The animal is an ovine. Preferred Method: In (M), inhibiting binding is through the C-terminus of %myostatin%. The Activin receptor is Act RIIA or Act RIIB. ACTIVITY - None given. MECHANISM OF ACTION - Muscle mass increaser. From pronuclear injections of this construct, a total of 7 founder animals positive for the transgene were identified. Analysis of these founder animals at 7 months of age revealed that all seven had significant increase in skeletal muscle mass with individual muscles of these founder animals weighing up to 125 % more than those of control non-transgenic animals derived from similar injections. The increases in muscle weights in these founder animals resulted from the expression of the transgene. All muscles of both male and female mice from the C5 line weighed 30-60 % more than those of control animals, whereas all muscles from C11 mice weighed 110-180 % more. The relative levels of transgene expression was correlated with the relative magnitude of the increase in muscle weights. For e.g. animals from the C11 line, which had the greatest increases in muscle weights, also had the highest levels of transgene expression. Expression of a dominant negative form of Act RIIB can cause increases in muscle mass. In order to determine whether expression of dominant negative Act RIIB also caused both hyperplasia and hypertrophy, sections of the gastrocnemius and plantaris muscles of animals from the C27 line were analyzed. Compared to control muscles, the muscles of the C27 animals showed a clear increase in overall cross-sectional area. This increase in area resulted partially from an increase in fiber number. The gastrocnemius and plantaris muscles had a total of 10015+1143 fibers in animals from the C27 line (n=3) compared to 7871+364 fibers in control animals (n=3). USE - (I) is useful for tissue-specific expression of follistatin in a transgenic animal, by expressing (I) in the cells of a transgenic animal, where the expression cassette is integrated into the genome of the animal, where the cassette is expressed to result in elevated levels of follistatin in the animal, thus resulting in increased muscle mass in the transgenic animal relative to a corresponding nontransgenic animal. (I) is useful for producing a chimeric non-human animal, by obtaining an ovum from animal ovaries, maturing the ovum in vitro, fertilizing the mature ovum in vitro to form a zygote, introducing into the zygote in vitro a nucleic acid construct comprising in operable association a DNA sequence encoding a truncated activin Type II receptor, a %myostatin% propeptide, or follistatin, and a regulatory sequence that promotes expression of the DNA sequence encoding the polypeptide, maturing the zygote to a preimplantation stage embryo in vitro, and transplanting the embryo into a recipient female animal, where the female animal gestates the embryo to produce a chimeric animal. (All claimed.) TA is useful for identifying agents for enhancing muscle growth for therapeutic purposes and agriculture applications. ADMINISTRATION - Anti-%myostatin% receptor monoclonal %antibody% is administered at a dose of 0.1 micro-g/kg-100 mg/kg, preferably 10-50 mg/kg, through intravenous, intramuscular or subcutaneous route. Pharmaceutical composition is administered through oral, topical, nasal, parenteral (intravenous, intramuscular, subcutaneous, intraorbital, intracapsular, intraperitoneal, intrarectal, or intracisternal route. EXAMPLE - DNAs encoding a truncated form of murine Activin Type II receptor (Act RIIB), the murine %myostatin% pro peptide, and the human follistatin short form were cloned into the MDAF2 vector containing the myosin light chain promoter and 1/3 enhancer. Purified transgenes including the myosin light chain regulatory sequences and SV40 processing sites were used for microinjections. Transgenic founders in a hybrid SJL/C57BL/6 background were mated to wild type C57BL/6 mice, and all studies were carried out using F1 offspring. For analysis of muscle weights, individual muscles were dissected from both sides of nearly all animals, and the average of the left and right muscle weights was used. Analysis of fiber numbers and sizes, and RNA isolation and Northern analysis were also carried out using the offspring. (188 pages)

2/7/86 (Item 20 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0304250 DBR Accession No.: 2003-06035 PATENT
Novel %myostatin% immunoconjugate useful for altering the phenotype of animals, e.g. avians, comprises full length %myostatin% polypeptide linked to a carrier - the use of human protein for vaccine and animal protection
AUTHOR: EL HALAWANI M E; YOU S
PATENT ASSIGNEE: EL HALAWANI M E; YOU S 2002
PATENT NUMBER: US 20020127234 PATENT DATE: 20020912 WPI ACCESSION NO.: 2003-039572 (200303)
PRIORITY APPLIC. NO.: US 754826 APPLIC. DATE: 20010104
NATIONAL APPLIC. NO.: US 754826 APPLIC. DATE: 20010104
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A %myostatin% immunoconjugate (I) comprising a full length %myostatin% polypeptide linked to a carrier, is new. DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for a vaccine or immunogenic composition (II) comprising (I). BIOTECHNOLOGY - Preferred Immunoconjugate: (I) comprises a vertebrate %myostatin% polypeptide or an avian %myostatin% polypeptide, preferably a turkey %myostatin% polypeptide comprising a sequence of 109 amino acids fully defined in the specification. (I) comprises a fusion polypeptide. The carrier is keyhole limpet hemocyanin. Preferred Composition: (II) comprises a pharmaceutically acceptable excipient. ACTIVITY - None given. MECHANISM OF ACTION - Vaccine (claimed); Modulator of endogenous %myostatin% activity in a vertebrate. To enhance its immunogenicity %myostatin% was conjugated to a carrier before being introduced into turkey hens. The mature coding region of turkey %myostatin% DNA (372 base pairs encoding 109 amino acid residues) was subcloned into the PQE expression vector system using Escherichia coli strain M15 (pREP4) and SG13009 (pREP4). His-tag fusion proteins were purified. The His-tag %myostatin% fusion protein was conjugated to keyhole limpet hemocyanin (KLH) and used to immunize 1st cycle breeder hens. The hens were inseminated weekly. On day 1 of the vaccination schedule, hens were vaccinated with 125 microg of the immunogen in Freund's complete adjuvant. At thirty-six days post-vaccination hens were light stimulated. Nine and sixteen days later, a blood sample was taken from 20 hens per treatment. A first booster (25 microg in Freund's incomplete adjuvant) was given sixteen days after light stimulation. Ten days later, egg collection began. Subsequent blood samples were drawn at 4, 18, 32, 49, 59, 74, and 88 days after the egg collection began. Subsequent boosts were at 18, 49, and 74 days relative to the egg collection. Titers were determined at 4 and 59 days after the first egg collection. In the first study, 8 hens were immunized with KLH and 32 with KLH-%myostatin%. Anti-%myostatin% %antibody% levels in %myostatin%-immunized poults were higher than KLH-immunized poults for at least four weeks after hatching. In the second study, the body and organ weights for progeny of %myostatin%- and KLH-immunized birds was determined. The progeny of %myostatin%-immunized birds had increased body weight, as well as increased heart, breast muscle and thigh muscle weight, and a decrease in abdominal fat weight. The increase in body weight was still evident in 6 week old birds from %myostatin%-immunized hens relative to controls. USE - (II) is useful for increasing muscle mass in progeny of an egg-laying vertebrate, preferably an avian such as turkey, by administering to the egg-laying vertebrate or to an egg of the vertebrate (II) and obtaining progeny of the vertebrate which have increased muscle mass relative to a progeny from a corresponding vertebrate which was not administered (II). The muscle mass in progeny from the vaccinated vertebrate is increased by 5%. Progeny from the vaccinated vertebrate have an increase in body weight, a reduction in body fat content, an increase in testis size, or its combination, an increase in muscle mass, or an increase in breast or thigh muscle weight. (II) is useful to passively immunize progeny of a female vertebrate, by administering (II) to the vertebrate prior to, during or after fertilization, or any of its combination, of at least one egg of the vertebrate, and obtaining progeny from the fertilized egg which comprise anti-%myostatin% %antibodies%. (II) is useful to decrease body fat in a vertebrate, by administering to a female vertebrate (II), and obtaining progeny of the female vertebrate which have decreased body fat relative to a progeny of a corresponding female vertebrate which was not administered (II). (II) is useful to increase testis size in a vertebrate, by administering to a female vertebrate (II), and obtaining male progeny of the female vertebrate which have an increase in testes size relative to a male progeny of a corresponding female vertebrate which was not administered (II), or by administering (II) to the vertebrate so as to result in the vertebrate having an increase in testes size. The vertebrate is a livestock animal such as pig or an egg-laying vertebrate such as turkey or chicken (all claimed). (I) is useful in turkey and swine production. (II) is useful for increasing the number or size of muscle cells, increasing the muscle strength, increasing appetite or feed uptake, or for increasing the lifespan of a vertebrate. ADMINISTRATION - (II) is administered at a dose of 0.01 microg-300 mg, preferably 5 microg-50 mg, by parenteral or oral route. EXAMPLE - %Myostatin% peptides, e.g. (S1), (S2) or (S3), were prepared. Each received a peptide immunogen and the sequences were chosen based on relative hydrophilicity/hydrophobicity. The oligopeptides were coupled to the carrier protein keyhole limpet hemocyanin (KLH) using the method of Lemer et al. (1981). 5 g of Sephadex G-25 was equilibrated with 0.05 M sodium phosphate for about 3 hours at 25 degrees Centigrade. KLH was dissolved in 0.05 sodium phosphate. Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) was dissolved in N,N-dimethyl formamide. 200 microl of KLH (4 mg) and 10 microl MBS (0.5 mg) was combined and incubated with gentle vortexing for 30 minutes at room temperature. The mixture was subjected to centrifugation and the supernatant was loaded onto a 10 ml G-25 column. The sample was eluted with 0.05 M sodium phosphate and 12 drop fractions were collected. Fractions absorbing at OD280 were pooled. The peptide was solubilized in a minimal amount of water and mixed with KLH-MBS in a 1:1 ratio. Sodium phosphate 0.05 M was added to a final concentration of 1.25 mg/ml peptide. The mixture was incubated for 3 hours at room temperature with constant shaking. The product was stored at -80 degrees Centigrade. Asn-Met-Leu-Thr-Phe-Asn-Gly-Lys-Glu-Gln-Ile-Ile-Thr -Gly-Lys-Ile (S1) Asp-Cys-Asp-Glu-His-Ser-Thr-Glu-Ser-Arg-Cys (S2) Ile-Ala-Pro-Lys-Arg-Thr-Lys-Ala-Asn (S3)(40 pages)

2/7/87 (Item 21 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0302380 DBR Accession No.: 2003-04165
Inhibition of %myostatin% in adult mice increases skeletal muscle mass and strength - %antibody% production against protein and cell culture for use in disease therapy
AUTHOR: WHITTEMORE LA; SONG KN; LI XP; AGHAJANIAN J; DAVIES M; GIRGENRATH S; HILL JJ; JALENAK M; KELLEY P; KNIGHT A; MAYLOR R; O'HARA D; PEARSON A; QUAZI A; RYERSON S; TAN XY; TOMKINSON KN; VELDMAN GM; WIDOM A; WRIGHT JF; WUDYKA S; ZHAO L; WOLFMAN NM
CORPORATE AFFILIATE: Wyeth Ayerst Res
CORPORATE SOURCE: Whittemore LA, Wyeth Ayerst Res, Musculoskeletal Sci Dept, 200 Cambridge Pk Dr, Cambridge,MA 02140 USA
JOURNAL: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS) 300, 4, 965-971
ISSN: 0006-291X
LANGUAGE: English
ABSTRACT: AUTHOR ABSTRACT - A human therapeutic that specifically modulates skeletal muscle growth Would potentially provide a benefit for a variety of conditions including sarcopenia, cachexia, and muscular dystrophy. %Myostatin% , a member of the TGF-beta family of growth factors, is a known negative regulator of muscle mass, as mice lacking the %myostatin% gene have increased muscle mass. Thus, an inhibitor of %myostatin% may be useful therapeutically as an anabolic agent for muscle. However, since %myostatin% is expressed in both developing and adult muscles, it is not clear whether it regulates muscle mass during developmentor in adults. In order to test the hypothesis that %myostatin% regulates muscle mass in adults, we generated an inhibitory

%antibody% to %myostatin% and administered it to adult mice. Here we show that mice treated pharmacologically with an %antibody% to %myostatin% have increased skeletal muscle mass and increased grip strength. These data show for the first time that %myostatin% acts postnatally as a negative regulator of skeletal muscle growth and suggest that %myostatin% inhibitors could provide a therapeutic benefit in diseasesfor which muscle mass is limiting. (C) 2002 Elsevier Science (USA). All rights reserved. DERWENT ABSTRACT: For monoclonal %antibody% preparation, %myostatin% knockout mice were immunized with recombinant %myostatin% purified from CHO cell conditioned media and hybridoma cells were generated using standard techniques. Hybridoma cells secreting anti-%myostatin% %antibodies% were identified by solid and solution phase ELISA to recombinant %myostatin%. Standard ELISA techniques and a pGL3-(CAGA)12 reporter assay were used to determine the IC50 with which selected %antibody% clones inhibited the binding of %myostatin% to its receptor, ActRIIB(7 pages)

2/7/88 (Item 22 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0289489 DBR Accession No.: 2002-11336 PATENT
New growth differentiation factor (GDF) receptors and modulators, useful for ameliorating wasting disorders such as cachexia, muscular dystrophy or neuromuscular disease or a metabolic disorder such as obesity or type II diabetes - vector-mediated gene transfer and expression in CHO cell culture, antisense oligonucleotide, %antibody% and %myostatin% antagonist for use in cachexia, anorexia, muscular dystrophy, neuromuscular disease, metabolic disorder,obesity and diabetes therapy and peptidomics
AUTHOR: LEE S; MCPHERRON A C
PATENT ASSIGNEE: UNIV JOHNS HOPKINS SCHOOL MEDICINE 2002
PATENT NUMBER: WO 200210214 PATENT DATE: 20020207 WPI ACCESSION NO.: 2002-217116 (200227)
PRIORITY APPLIC. NO.: US 626896 APPLIC. DATE: 20000727
NATIONAL APPLIC. NO.: WO 2001US23615 APPLIC. DATE: 20010726
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A substantially purified growth differentiation factor (GDF) receptor or its functional peptide portion, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a substantially purified polynucleotide encoding the GDF receptor polypeptide or a functional portion of the GDF receptor polypeptide;(2) an oligonucleotide that specifically hybridizes to the polynucleotide above, where the oligonucleotide is at least 15 nucleotides in length; (3) a recombinant host cell containing the polynucleotide; (4) an %antibody% that specifically binds the GDF receptor or its functional peptide portion; (5) a virtual representation of the GDF receptor or its functional peptide portion; (6) modulating an effect of %myostatin% on a cell by contacting the cell with an agent that affects %myostatin% signal transduction in the cell; (7) ameliorating the severity of a pathologic condition characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject, comprising modulating %myostatin% signal transduction in a muscle cell or an adipose tissue cell in the subject; (8) modulating the growth of muscle tissue or adipose tissue in a eukaryotic organism by administering to the organism an agent that affects signal transduction by a GDF receptor in the organism; (9) identifying an agent that specifically interacts with a GDF receptor or its functional peptide portion comprising: (a) contacting a GDF receptor with a test agent and determining that the test agent specifically interacts with the GDF receptor, thus identifying an agent that specifically interacts with a GDF receptor; or(b) testing, in a computer system, a virtual test agent for the ability to interact specifically with a virtual GDF receptor its functional peptide portion and detecting a specific interaction of the virtual test agent with the virtual GDF receptor or its functional peptide portion, thus identifying an agent that interacts specifically with the GDF receptor or its functional peptide portion; and (10) identifying a GDF receptor comprising: (a) contact a GDF or its functional peptide portion, and a sample suspected of containing a GDF receptor under conditions suitable for the GDF to specifically interact with a receptor for the GDF; and (b) detecting a specific interaction of the GDF with a GDF receptor in the sample, thus identifying a GDF receptor. BIOTECHNOLOGY - Preferred Polypeptide: The GDF receptor consists of GDF-8 receptor or a GDF-11 receptor. The GDF receptor or its functional peptide portion has a dominant negative GDF receptor activity. The GDF receptor is a %myostatin% receptor. In method (6), the agent alters a specific interaction of %myostatin% receptor expressed by the cell. The %myostatin% receptor is an activin receptor, particularly an activin type IIB receptor. The agent comprises a peptide. In particular, the peptide comprises an extracellular domain of a %myostatin% receptor, which can specifically interact with %myostatin%, thus reducing or inhibiting %myostatin% signal transduction in the cell. The peptide also comprises a GDF prodomain or its functional peptide portion, which can specifically interact with %myostatin% to reduce or inhibit %myostatin% signal transduction in the cell. The GDF prodomain is a %myostatin% prodomain, which comprises 20-262 amino acid residues of a promyostatin polypeptide. Preferably, the %myostatin% prodomain comprises an amino acid sequence having amino acid residues: (a) 20-263 of the 376-amino acid sequence from Mus musculus; (b) 20-262 of the 375-amino acid sequence from Homo sapiens; (c) 20-262 of the 375-amino acid sequence from baboon; (d) 20-262 of the 375-amino acid bovine sequence; (e) 20-262 of the 374-amino acid sequence from Gallus gallus; (f) 20-263 of the 376-amino acid sequence from Rattus norvegicus; (g) 20-262 of the 375-amino acid sequence from Meleagris gallopavo; (h) 20-262 of the 375-amino acid porcine sequence; (i) 20-262 of the 375-amino acid ovine sequence; (j) 20-262 of the 374-amino acid sequence from Danio rerio; or (k) the functional peptide portion of (a)-(j). The %myostatin% prodomain further comprises a %myostatin% signal peptide. The peptide may be a mutant promyostatin polypeptide that is resistant to proteolytic cleavage at a proteolytic cleavage site comprising the amino acid sequence: Arg-Xaa-Xaa-Arg. In particular, the mutant %myostatin% polypeptide comprises a mutation in the amino acid sequence: Arg-Xaa-Xaa-Arg. The peptide specifically interacts with a %myostatin% receptor expressed by a cell, but does not activate %myostatin% signal transduction. The peptide is an anti-%myostatin% receptor %antibody%, an anti-idiotypic %antibody% of an anti-%myostatin% %antibody%, or a mutant %myostatin% receptor. The peptide specifically interacts with a %myostatin% receptor expressed by a cell, and activates %myostatin% signal transduction. The agent is follistatin or a polynucleotide. In method (6), contacting comprises introducing the polynucleotide into the cell. The %myostatin% receptor comprises a %myostatin% receptor extracellular domain, which binds %myostatin% , but with the proviso that it does not activate mystatin signal transduction. The mutant %myostatin% receptor comprises a membrane anchoring domain operatively associated with the %myostatin% receptor extracellular domain. The membrane anchoring domain comprises at least a membrane anchoring portion of a %myostatin% receptor transmembrane domain. Preferably, the mutant %myostatin% receptor lacks protein kinase activity. The peptide affects the level or activity of an intracellular polypeptide in a %myostatin% signal transduction pathway. The intracellular polypeptide is a Smad polypeptide, where the peptide is a dominant negative Smad polypeptide. The dominant negative Smad polypeptide lacks a phosphorylation site, where the Smad polypeptide consists of Smad 2, Smad 3 or Smad 4, and where method (6) comprises reducing or inhibiting %myostatin% signal transduction in the cell. The dominant negative Smad may also be Smad 6 or Smad 7, where method (6) comprises increasing %myostatin% signal transduction in the cell. The intracellular polypeptide is a c-ski polypeptide, where method (6) comprises reducing or inhibiting %myostatin% signal transduction. The polynucleotide is an antisense nucleotide sequence or encodes an antisense nucleotide sequence. Preferably, the antisense nucleotide sequence is an antisense c-ski nucleotide sequence, and method (6) comprises increasing %myostatin% signal transduction. The antisense nucleotide sequence is preferably an antisense Smad nucleotide sequence. The agent affects %myostatin% signal transduction, thus modulating the growth of muscle tissue or adipose tissue in the organism. The agent alters the specific interaction of %myostatin% with a %myostatin% receptor, this affecting %myostatin% signal transduction.

The agent also reduces or inhibits the specific interaction of %myostatin% with a %myostatin% receptor, this increasing the growth of the organism. The agent may be a polynucleotide, and the step of administering comprises introducing the polynucleotide into a cell in the organism. The polynucleotide encodes a peptide that is expressible in the cell. The GDF receptor may also be a %myostatin% receptor, where the agent is a %myostatin% receptor agonist, which increases %myostatin% signal transduction. In particular, the %myostatin% receptor agonist is a functional peptide portion of %myostatin% or an %antibody%. The agent may also be a %myostatin% antagonist, which reduces or inhibits %myostatin% signal transduction, where the %myostatin% receptor antagonist is a mutant promyostatin polypeptide. The test agent also comprises a combinatorial library of test agents comprising peptides, polynucleotides, peptidomimetics, small organic molecules, or combinations of these. Preferred Method: Method (7) comprises reducing or inhibiting %myostatin% signal transduction in a muscle cell, or increasing %myostatin% signal transduction in an adipose tissue cell. The method may involve increasing the growth of muscle tissue or decreasing the growth of adipose tissue, or both in the organism. The method may also comprise decreasing the growth of muscle tissue or increasing the growth of adipose tissue or both in the organism. Alternatively, the method may involve increasing the growth or muscle tissue or decreasing the growth of adipose tissue, or both in the organism. The eukaryotic organism is a vertebrate organism (e.g. bovine, ovine, porcine, canine, feline, murine or piscine) or an invertebrate organism (such as mollusk, e.g. shrimp, scallop, squid, octopus or snail). The method comprises administering to the mollusk the agent that reduces or inhibits the specific interaction of %myostatin% with a %myostatin% receptor, thus increasing the growth of the mollusk. The method may also comprise administering the agent that increases mysotatin signal transduction, thus decreasing the growth of muscle tissue in the mollusk, particularly a slug. In method (10) , the GDF is %myostatin% and the sample is a muscle cell sample. Preferred Virtual Representation: The virtual representation comprises an agent that interacts specifically with the GDF receptor. Preferred Polynucleotide: The polynucleotide is preferably contained in a vector. The GDF receptor or its functional peptide portion, which encoded by the polynucleotide, is expressed in the recombinant host cell. ACTIVITY - Immunomodulator; antidepressant; anorectic; neuroprotective; antidiabetic. MECHANISM OF ACTION - Growth differentiation factor modulator. The inguinal, epididymal and retroperitoneal fat pads in male mice were examined. There was no difference in the weights of any of these fat pads between wild type and mutant mice at two months of age. By 5 to 6 months age, wild type and heterozygous knock-out mice both exhibited a large range of fat weights, and on average, fad pad weights increased by approximately 3-fold to 5-fold by the time the animals reached 9 to 10 months of age. Due to the large range of fat pad weight observed in these animals, some animals showed as much larger increase (up to 10-fold) than others. In contrast to the wild type and heterogeneous knock-out mice, the fat pad weight of %myostatin% homozygous mutant mice were in a relatively narrow range and were virtually identical in 2 month old mice and in 9 to 10 month old mice. Thus, the increased fat accumulation that occurred with aging in the wild type mice was not observed in the homozygous %myostatin% knock-out mice. USE - The method and the receptor are useful for ameliorating the severity of a pathologic condition characterized by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject, particularly a wasting disorder (e.g. cachexia, anorexia, muscular dystrophy or neuromuscular disease) or a metabolic disorder (e.g. obesity or type II diabetes) (all claimed). ADMINISTRATION - Administration may be oral or parental (e.g. intravenous, intramuscular, subcutaneous, intraorbital, intracapsular, intraperitoneal, intrarectal or intracisternal). Dosage is about 0.1 Tg/kg - 100 mg/kg, preferably about 10-50 mg/kg. EXAMPLE - Stable Chinese hamster ovary (CHO) cell lines producing high levels of %myostatin% protein were generated by co-amplifying a %myostatin% expression cassette with a dihydrofolate reductase cassette using a methotrexate selection scheme. %Myostatin% was purified from the conditioned medium of the highest producing line by successive fractionation on hydroxyapatite, lentil lectin SEPHAROSE, diethylaminoethyl (DEAE), agarose, and heparin SEPHAROSE. Silver stain analysis revealed that the purified protein obtained following these four column chromatography steps consisted of two species with molecular masses of approximately 35 kilodaltons (kDA) and 12 kDA. (184 pages)

2/7/89 (Item 23 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0288160 DBR Accession No.: 2002-10007 PATENT
Novel substantially purified promyostatin polypeptide portion (%myostatin% prodomain or mature %myostatin% peptide), useful as %myostatin% signal transduction modulator in muscle cell or adipose tissue, for treating obesity - transgenic animal, %antibody%, drug screening, agonist, antagonist and antisense DNA useful for gene therapy and improved foodstuff
AUTHOR: LEE S; MCPHERRON A C
PATENT ASSIGNEE: UNIV JOHNS HOPKINS SCHOOL MEDICINE 2002
PATENT NUMBER: WO 200209641 PATENT DATE: 20020207 WPI ACCESSION NO.: 2002-179989 (200223)
PRIORITY APPLIC. NO.: US 628112 APPLIC. DATE: 20000727
NATIONAL APPLIC. NO.: WO 2001US23510 APPLIC. DATE: 20010726
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A purified promyostatin polypeptide portion (I), is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a mutant promyostatin polypeptide (II), comprising a mutation which disrupts proteolytic cleavage site comprising an amino acid sequence (S1); (2) a purified polynucleotide (III) encoding (I); (3) a polynucleotide (IV) encoding (II); (4) a transgenic non-human organism comprising (III) or (IV); (5) an %antibody% (V) that specifically binds (I); (6) a virtual representation of a promyostatin polypeptide, or its functional peptide portion; and (7) identifying (M1) a functional peptide portion of a %myostatin% prodomain that interacts specifically with a %myostatin% peptide, comprising testing a peptide portion of a %myostatin% prodomain for the ability to interact specifically with a %myostatin% peptide, and detecting a specific interaction of the peptide portion with the %myostatin% peptide, thereby identifying a functional peptide portion of the %myostatin% prodomain. (S1) is Arg-Xaa-Xaa-Arg. WIDER DISCLOSURE - Disclosed as new are the following: (1) purified peptide portion of growth differentiation factor (GDF)-11 polypeptide i.e. proteolytic fragment of pro-GDF-11 polypeptide or functional peptide portion of such fragments; (2) mutant pro-GDF-11 polypeptide portion; (3) polynucleotide encoding pro-GDF-11 polypeptide portion; (4) identifying a functional peptide portion of GDF-11 prodomain that interacts specifically with GDF-11 peptide; (5) %antibodies% that can specifically bind to peptide portion of pro-GDF-11 polypeptide; (6) a kit containing a peptide portion of promyostatin or pro-GDF-11 polypeptide, or a mutant promyostatin or pro-GDF-11 polypeptide, or a polynucleotide encoding peptide portion of promyostatin or a pro-GDF-11 polypeptide, or an %antibody% that specifically binds to such a peptide portion of promyostatin or a pro-GDF-11 polypeptide or a combination of the components; (7) identifying small organic molecules that mimic the action of GDF prodomain, reducing or inhibiting %myostatin% or GDF-11 signal transduction; (8) identifying agents that specifically interact with a GDF receptor for example an Act RIIA, Act RIIB or other GDF receptor, the agents being useful as GDF receptor agonists or antagonists; (9) polynucleotide (VI) encoding a GDF receptor or its functional peptide portion; (10) vectors comprising (III), (IV), (VI); (11) %antibodies% that bind to promyostatin polypeptide and thus useful for reducing or inhibiting proteolytic cleavage of promyostatin to mature %myostatin% peptide (referred as %myostatin%); (12) %antibody% that specifically binds to GDF receptor or its functional peptide portion; (13) modulating %myostatin% signal transduction in a cell by using a suitable agent; (14) identifying an agent that alters the effect of GDF such as %myostatin% on a cell by screening for agents that can competitively or non-competitively inhibit, mediate or enhance %myostatin% binding to the receptor; (15) pharmaceutical compositions

containing an agent useful for modulating %myostatin% signal transduction in a cell; (16) modulating growth of muscle tissue or adipose tissue by modulating signal transduction from a GDF receptor; (17) food products produced by transgenic organisms, which have increased nutritional value because of the increase in muscle tissue due to inhibition of %myostatin% activity; (18) a GDF receptor such as %myostatin% receptor (an activin type II receptor) which specifically interacts with %myostatin% and with GDF-11; (19) a recombinant cell that expresses GDF receptor polypeptide; (20) peptide portions of GDF receptor and polynucleotides encoding them; (21) identifying a GDF receptor polypeptide by screening genomic or cDNA libraries; (22) agents that interact with GDF receptor which are identified by a screening method for the agents; (23) non-human transgenic animals that have a phenotype characterized by expression of a GDF receptor; (24) GDF receptor polynucleotides that are degenerate from the above mentioned GDF receptor polynucleotide sequences; (25) oligonucleotide portions of polynucleotide encoding GDF receptor; (26) polynucleotides encoding mutant GDF receptor and mutant GDF receptor polypeptides; (27) a host cell/expression vector systems used to express a GDF receptor coding sequence; (28) producing animal food products having increased muscle content by introducing a transgene comprising an antisense molecule that is specific for a polynucleotide encoding a %myostatin% receptor; (29) GDF receptor variants; (30) identifying a composition that binds to a GDF receptor; and (31) identifying chemical compounds that bind to GDF receptors. BIOTECHNOLOGY - Preferred Peptide: (I) is a portion of a vertebrate promyostatin polypeptide. The vertebrate promyostatin is a human, murine, chicken, rat, baboon, bovine, turkey, porcine, ovine, zebrafish, salmon allele 2, salmon allele 2 promyostatin polypeptide having a 375 (S2), 375 (S4), 374 (S8), 376 (S6), 375 (S10), 375 (S12), 375 (S18), 375 (S14), 375 (S16), 374 (S20), 157 (S27), or 136 (S29) residue amino acid sequence, all fully defined in the specification, respectively. (I) is a proteolytic fragment of a promyostatin polypeptide, or its functional peptide portion, where the proteolytic fragment or its functional peptide portion has any one of the following activity: %myostatin% signal transduction stimulatory or inhibitory activity, %myostatin% binding activity, promyostatin binding activity, cellular localization activity, or a combination of any of the above mentioned activities. Preferably, (I) is produced by the cleavage of promyostatin polypeptide at a proteolytic cleavage site which comprises the amino acid sequence Arg-Xaa-Xaa-Arg. The proteolytic cleavage site comprises the amino acid sequence corresponding to residues 263-266 of (S2). (I) produced by the cleavage of promyostatin polypeptide, is: (a) %myostatin% prodomain comprising amino acid residues 20-262 of promyostatin polypeptide, or its functional peptide portion, where preferably the %myostatin% prodomain comprises residues 20-263 of (S4) or (S6), or residues 20-262 of (S2), (S10), (S12), (S8), (S18), (S14), (S16), (S20) or its functional peptide portion; (b) %myostatin% prodomain comprising residues 1-44 of (S27) or residues 1-23 of (S29); (c) mature %myostatin% which comprises amino acid residues 268-374 of a promyostatin polypeptide, or its functional peptide portion, where the mature %myostatin% comprises 268-375 of (S4) or (S6), or residues 267-374 of (S2), (S10), (S12), (S8), (S18), (S14), (S16), (S20) or its functional peptide portion; or (d) mature %myostatin% comprising residues 49-157 of (S27), 28-136 of (S29) or a functional peptide portion of the amino acid sequence. The %myostatin% prodomain comprising residues 20-262 or 263 of (S2), (S10), (S12), (S8), (S18), (S14), (S16), (S20), further comprises amino acid residues 1-20 of promyostatin polypeptide having a sequence of (S2), (S4), (S6), (S10), (S12), (S8), (S18), (S14), (S16), (S20), (S27) or (S29). Preferred Polypeptide: (II) comprises a mutation of an arginine residue of the proteolytic cleavage site. Preferred Polynucleotide: (III) encodes prodomain of the promyostatin polypeptide further comprising a promyostatin signal peptide or functional peptide portion of the prodomain. Preferred %Antibody% : (V) specifically binds a prodomain of the promyostatin polypeptide or its functional peptide portion. Preferred Method: The testing is performed in a computer system using a virtual peptide portion of a promyostatin prodomain and a virtual %myostatin% peptide. The testing comprises contacting the peptide portion of %myostatin% prodomain and the %myostatin% peptides under conditions suitable for a %myostatin% prodomain to specifically interact with the %myostatin% peptide. ACTIVITY - Neuroprotective; Anorectic; Antidiabetic; Immunomodulator; Antiatherosclerotic; Hypotensive; Cardiant; Metabolic. Chinese hamster ovary (CHO) cells that express %myostatin% were injected into nude mice. The nude mice that had %myostatin% expressing CHO cell tumors showed severe wasting over the course of 12-16 days following injection of the cells. White fat pad weights (intrascapular white, uterine, and retroperitoneal fat) were reduced by greater than 90 % compared to mice bearing control CHO cell tumors. Muscle weights were also severely reduced, with individual muscles weighting half as much in %myostatin% expressing mice as in control mice by day 16. This loss in muscle weight was reflected by a corresponding decrease in fiber sizes and protein content. The weight loss and hypoglycemia were not due to a difference in food consumption, as all of the mice consumed equivalent amounts of food at each time interval examined during the 16 day course of the study. MECHANISM OF ACTION - %Myostatin% activity modulator; %myostatin% signal transduction in muscle cell or adipose tissue, modulator. USE - (I) (a %myostatin% peptide) is useful as a target for treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis or muscular dystrophy. (I) (a %myostatin% prodomain) inhibits mysostatin signal transduction, while (I) (mature %myostatin% peptide referred as %myostatin%) is useful for inducing %myostatin% signal transduction by interacting specifically with %myostatin% receptor expressed on the surface of the cell. Modulating %myostatin% signal transduction is useful for regulating skeletal muscle mass, where (I) is a negative regulator or muscle growth. Modulating %myostatin% signal transduction in a muscle cell or adipose tissue is useful for treating pathological conditions associated with %myostatin% such as obesity and type II diabetes, cachexia, conditions associated with obesity, e.g. atherosclerosis, hypertension, myocardial infarction, muscle wasting disorders such as muscular dystrophy, neuromuscular disorders, cachexia, or anorexia. (I) (%myostatin% prodomain) or (II) is useful for modulating the growth of muscle or adipose tissue in an organism. (I) (%myostatin% prodomain) is useful for increasing muscle mass or reducing fat content of an organism which is useful as a food source, and (I) (%myostatin% peptide is useful for decreasing the growth of muscle tissue in an organism e.g. an organism detrimental to an environment. (II) which has dominant negative activity with respect to %myostatin% or growth differentiation factor (GDF)-11 is useful for reducing or inhibiting %myostatin% signal transduction. EXAMPLE - To elucidate the biological activity of %myostatin%, large quantities of %myostatin% protein were purified for bioassays. Stable Chinese hamster ovary (CHO) cell lines producing high levels of %myostatin% protein were generated by co-amplifying a %myostatin% expression cassette with a dihydrofolate reductase cassette using a methotrexate selection scheme. %Myostatin% was purified from the conditioned medium of the highest producing line by successive fractionation on hydroxyapatite, lentil lectin SEPHAROSE (RTM), diethylaminoethyl (DEAE) agarose, and heparin SEPHAROSE (RTM). Silver stain analysis revealed that the purified protein obtained following these four column chromatography steps consisted of two species with molecular masses of 35 kDa and 12 kDa. The purified protein preparation was determined by various criteria to represent a complex of two %myostatin% prodomain peptides and a disulfide-linked dimer of mature C-terminal %myostatin% peptides. Western blot analysis using %antibodies% raised against specific portions of the promyostatin sequence, identified the 35 kDa band as the prodomain and the 12 kDa band as the mature C-terminal peptide. Second, under non-reducing conditions, the species reacting with %antibodies% directed against the mature C-terminal peptide had an electrophoretic mobility consistent with a disulfide linked dimer. Third, the molar ratio of prodomain to mature C-terminal peptide was 1:1. Fourth, the prodomain and mature C-terminal peptide copurified through the four column chromatography steps. Finally, the mature C-terminal peptide bound to the lentil lectin column even though the C-terminal region did not contain N-linked glycosylation signals, indicating that the mature C-terminal peptide bound to the column due to its interaction with the prodomain peptide, which contained a potential N-linked glycosylation site. These results indicated that %myostatin% produced by the genetically modified CHO cells was secreted in a proteolytically processed form, and that the resulting prodomain

and mature C-terminal region associate non-covalently to form a complex containing two prodomain peptides and a disulfide-linked dimer of C-terminal proteolytic fragments. The heparin eluate, which consisted of a complex of prodomain and mature C-terminal peptide, was further purified using an high performance liquid chromatography (HPLC) reversed phase column. The C-terminal dimer eluted from the HPLC column earlier than the prodomain. Fractions that contained mostly prodomain also were obtained, although these fractions contained small amounts of the C-terminal dimer. Some of the protein also was present a higher molecular weight complexes.(175 pages)

2/7/90 (Item 24 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0264798 DBR Accession No.: 2001-04552 PATENT
Promoter which regulates expression of %myostatin% gene, whose inhibitors are useful for preventing conditions involving muscle wasting such as cancer, multiple sclerosis, aging and acquired immune deficiency syndrome - plasmid pGL-3-mediated luciferase reporter gene transfer, expresion in mammal host cell and monoclonal %antibody% for recombinant protein production, drug screening and disease therapy
AUTHOR: Wu-Wong J R; Wang J
CORPORATE SOURCE: Abbot Park, IL, USA.
PATENT ASSIGNEE: Abbott-Lab. 2000
PATENT NUMBER: WO 200077206 PATENT DATE: 20001221 WPI ACCESSION NO.: 2001-071272 (2008)
PRIORITY APPLIC. NO.: US 329685 APPLIC. DATE: 19990610
NATIONAL APPLIC. NO.: WO 2000US15868 APPLIC. DATE: 20000609
LANGUAGE: English
ABSTRACT: An isolated promoter (I) which induces expression of the %myostatin% gene (e.g. from human) and having a sequence of 3,438 bp (specified), is claimed. Also claimed are: a vector (II, e.g. plasmid pGL-3 enhancer vector) containing (I) and a DNA sequence encoding a reporter molecule (e.g. luciferase) which is operably linked to (I); a host cell (III) containing (II); an %antibody% (IV) produced n response to immunization with %myostatin%; a composition (V) containing (IV); identifying a composition which inhibits activation of the %myostatin% promoter; identifying a composition which inhibits expression of %myostatin% using monoclonal or polyclonal %antibodies% ; and identifying a composition or mixture of compositions which prevents %myostatin% from binding to a %myostatin% receptor. (I) is useful for activating or regulating expression of the %myostatin% gene. Compositions identified by the methods are useful for inhibiting the activity of the %myostatin% promoter and for preventing conditions involving muscle wasting. The %antibodies% have therapeutic, veterinary and agricultural applications. (31pp)

2/7/91 (Item 25 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0243781 DBR Accession No.: 1999-14546 PATENT
New %myostatin% peptide, multimers and immunoconjugates for eliciting an immune response in a vertebrate against a %myostatin% immunogen - recombinant %myostatin% production via vector-mediated gene transfer and expression in transgenic animal and %antibody% for recombinant vaccine for muscle-wasting disease therapy
AUTHOR: Barker C A; Morsey M
CORPORATE SOURCE: Saskatoon, Saskatchewan, Canada.
PATENT ASSIGNEE: Biostar 1999
PATENT NUMBER: WO 9942573 PATENT DATE: 19990826 WPI ACCESSION NO.: 1999-527471 (1944)
PRIORITY APPLIC. NO.: US 75213 APPLIC. DATE: 19980219
NATIONAL APPLIC. NO.: WO 99CA128 APPLIC. DATE: 19990219
LANGUAGE: English
ABSTRACT: A %myostatin% peptide (I) which consists of 3-100 amino acids and contains at least epitope of %myostatin%, is new. Also claimed are: a %myostatin% peptide (II) consisting of 3-200 amino acids, derived from a %myostatin% region of amino acids 1-350 from mouse, rabbit, human, baboon, cattle, pig, sheep, fowl, turkey or zebrafish %myostatin%, preferably amino acids 1-275, 25-300, 50-325 or 75-350 (all specified); a %myostatin% multimer (III) which consists of 2 or more selected %myostatin% immunogens; a %myostatin% immunoconjugate (IV) containing at least one (I), (II) or (III) linked to an immunological carrier; a polynucleotide (V) encoding (I)-(IV); a recombinant vector containing (V) operably linked to a control element; a host cell transformed with the vector; the recombinant production of (I), (II), (III) or (IV); and an %antibody% specific for (I). (I)-(IV) may all be useful as vaccines for eliciting an immune response against a %myostatin% immunogen in a vertebrate. The recombinant vaccines may be useful for treating muscle-wasting conditions and modulating the activity of GDF11. The average weight of mice injected 3 times with 50 ug of the vaccines increased from 12.67 to 14.85. (107pp)

2/7/92 (Item 26 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0242515 DBR Accession No.: 1999-13280 PATENT
New differentiation factor useful for treating neurodegenerative diseases - plasmid pRSET-mediated GDF-8 antigen expression in BL21 cell, %antibody%, transgenic animal, agonist, antagonist and antisense oligonucleotide
AUTHOR: Lee S J; McPherron A C
CORPORATE SOURCE: Baltimore, MD, USA.
PATENT ASSIGNEE: Univ.Johns-Hopkins 1999
PATENT NUMBER: WO 9940181 PATENT DATE: 19990812 WPI ACCESSION NO.: 1999-494289 (1941)
PRIORITY APPLIC. NO.: US 124180 APPLIC. DATE: 19980728
NATIONAL APPLIC. NO.: WO 99US2511 APPLIC. DATE: 19990205
LANGUAGE: English
ABSTRACT: A cell growth differentiation factor-8 (GDF-8) and the polynucleotide encoding GDF-8 are new. Also claimed are: a method for producing animal food products having increased bone content; a method for producing bird, pig or cattle food products having an increased number of ribs; and treating a chronic or acute kidney disease in a subject by administering a reagent which affects GDF-8 activity or expression, such as an antagonist or agonist, an %antibody% or an antisense oligonucleotide. The GDF-8 is used in treating neurodegenerative diseases, muscular degenerative diseases or bone-associated disorders or disorders related to abnormal proliferation of adipocytes, tissue repair like kidney repair due to trauma and in maintaining cells or tissues in culture prior to transplantation. Transgenes which disrupt or interfere with expression of GDF-8 can be used to produce transgenic animals with increased muscle tissue and bone. These transgenic animals can be killed and used in food products. In an example %antibodies% were prepared by plasmid pRSET-mediated GDF-8 antigen expression in BL21 cell for immunization of rabbits and chickens. (129pp)

2/7/93 (Item 27 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0241538 DBR Accession No.: 1999-11639 PATENT
Increasing muscle mass in animals - by injection of a recombinant fusion protein, encoding a %myostatin%, carrier protein fusion protein
AUTHOR: Hickey G F
CORPORATE SOURCE: Rahway, NJ, USA.
PATENT ASSIGNEE: Merck-USA 1999
PATENT NUMBER: GB 2333706 PATENT DATE: 19990804 WPI ACCESSION NO.: 1999-397436 (1934)
PRIORITY APPLIC. NO.: US 73438 APPLIC. DATE: 19980202
NATIONAL APPLIC. NO.: GB 992041 APPLIC. DATE: 19990129
LANGUAGE: English
ABSTRACT: A means of increasing the muscle mass in animals is claimed. It

involves the administration of a vaccine that is able to induce the production of %myostatin%-specific %antibodies%, or administering an immunoneutralizing amount of %myostatin%-specific %antibodies%. The %antibodies% allow an increase in skeletal muscle mass in livestock animals, including cattle, sheep, pigs and fowl, thus increasing their value as food sources. The vaccine is preferably a %myostatin% epitope-carrier protein conjugate, in which the epitope is selected from the C-terminal functional domain of %myostatin%, and the carrier protein is e.g. diphtheria toxoid, tetanus toxoid, ovalbumin, keyhole limpet hemocyanin, etc. The vaccine may alternatively be a fusion protein produced by recombinant DNA technology. The vaccine is preferably administered by s.c. or intramuscular injection. The vaccine may also contain an adjuvant, used to enhance the immunological response, e.g. aluminum compound, or tocopherol acetate solubilizate. (10pp)

2/7/94 (Item 28 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2006 The Thomson Corp. All rts. reserv.

0240007 DBR Accession No.: 1999-10108 PATENT
Method for detecting the presence of a target %myostatin% variant - %myostatin% variant DNA probe and DNA primer construction and %antibody%, used for pig, cattle, bird, fish or horse increased muscle mass predisposition determination
AUTHOR: Lee S J; McPherron A C
CORPORATE SOURCE: Baltimore, MD, USA.
PATENT ASSIGNEE: Univ.Johns-Hopkins 1999
PATENT NUMBER: WO 9924618 PATENT DATE: 19990520 WPI ACCESSION NO.: 1999-337720 (1928)
PRIORITY APPLIC. NO.: US 967089 APPLIC. DATE: 19971110
NATIONAL APPLIC. NO.: WO 98US23850 APPLIC. DATE: 19981110
LANGUAGE: English
ABSTRACT: A new method for the detection of the presence of a target %myostatin% variant DNA sequence in a sample from a subject with increased muscle mass (or with a predisposition to increased muscle mass) when compared to a subject with a wild-type DNA sequence involves: contacting DNA isolated from the specimen with an agent that detects %myostatin% DNA; and detecting the presence of the target %myostatin% variant DNA sequence in the specimen, where the presence of the variant DNA sequence is indicative of a predisposition for increased muscle mass, or increased muscle mass. Also claimed are: a kit for detecting the target DNA containing a DNA probe specific for a defined DNA sequence and a means for detecting hybridization of the DNA probe and target DNA; and a diagnostic kit containing an %antibody% which binds to a specified portion of either the wild-type or variant %myostatin% protein. The method preferably involves a second stage of amplifying of the DNA using a DNA primer. Animals with increased muscle mass (e.g. pig, fish, horse, bird and cattle) are of importance in agriculture. (53pp)

2/7/95 (Item 29 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2006 The Thomson Corp. All rts. reserv.

0234212 DBR Accession No.: 99-04313 PATENT
Increasing muscle mass in mammals - vector-mediated sense or antisense %myostatin% gene transfer and expression in cattle transgenic animal, used to increase muscle mass
AUTHOR: Grobet L; Georges M; Poncelet D
CORPORATE SOURCE: Liege, Belgium.
PATENT ASSIGNEE: Univ.Liege 1999
PATENT NUMBER: WO 9902667 PATENT DATE: 990121 WPI ACCESSION NO.: 99-120869 (9910)
PRIORITY APPLIC. NO.: US 7761 APPLIC. DATE: 980115
NATIONAL APPLIC. NO.: WO 98IB1197 APPLIC. DATE: 980714
LANGUAGE: English
ABSTRACT: A new method of increasing muscle mass in mammals having %myostatin% -expressing muscle cells, involves administering a nucleic acid molecule (I) substantially complementary to at least a portion of cattle mRNA encoding %myostatin% and of sufficient length to reduce %myostatin% expression and therefore increase muscle mass. Also claimed are: %myostatin% with a specified 376 amino acid protein sequence; a method of increasing muscle mass in a mammal by administering a nucleic acid molecule with ribozyme activity and (I); a diagnostic kit for determining muscular hyperplasia in a mammal containing 2 DNA primers complementary to sequences upstream and downstream of a mutation in the %myostatin% gene; similar diagnostic kits for determining double muscling, %myostatin% genotype using DNA primers with specified DNA sequences; DNA probes and DNA primers used in the kits; a transgenic animal with a muscle hyperplasia phenotype; a cattle transgenic animal; and a transgenic mouse. The invention is used to increase muscle mass in an animal by administering a %myostatin% %antibody% , or administering an antisense sequence, and the transgenic cells can be used to produce %myostatin%. (49pp)

? b 411;set files allscience
07dec06 17:11:36 User219511 Session D666.4
$1.73 0.508 DialUnits File155
$6.16 28 Type(s) in Format 7
$6.16 28 Types
$7.89 Estimated cost File155
$1.98 0.330 DialUnits File5
$33.00 15 Type(s) in Format 7
$33.00 15 Types
$34.98 Estimated cost File5
$4.36 0.389 DialUnits File73
$71.30 23 Type(s) in Format 7
$71.30 23 Types
$75.66 Estimated cost File73
$24.93 1.118 DialUnits File357
$114.84 29 Type(s) in Format 7
$114.84 29 Types
$139.77 Estimated cost File357
OneSearch, 4 files, 2.344 DialUnits FileOS
$0.53 TELNET
$258.83 Estimated cost this search
$261.21 Estimated total session cost 3.200 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
(c) 2006 Dialog

*** DIALINDEX search results display in an abbreviated ***
*** format unless you enter the SET DETAIL ON command. ***
You have 297 files in your file list.
(To see banners, use SHOW FILES command)
? s gdf and (myo29 or myo029 or myo-29 or myo-029)

Your SELECT statement is:
s gdf and (myo29 or myo029 or myo-29 or myo-029)

Items	File
Examined 50 files	
1	107: Adis R&D Insight_1986-2006/Sep W1
1	128: PHARMAPROJECTS_1980-2006/Nov W3
Examined 100 files	
Examined 150 files	
2	340: CLAIMS(R)/US Patent_1950-06/Dec 05
8	349: PCT FULLTEXT_1979-2006/UB=20061130UT=20061123
2	357: Derwent Biotech Res.__1982-2006/Dec W2
2	399: CA SEARCH(R)_1967-2006/UD=14524
Examined 200 files	
Examined 250 files	
6	654: US Pat.Full._1976-2006/Dec 05

7 files have one or more items; file list includes 297 files.

? save temp; b 107,128,340,349,357,399,654;exs;rd
Temp SearchSave "TD343179174" stored
07dec06 17:12:47 User219511 Session D666.5
$3.67 1.387 DialUnits File411
$3.67 Estimated cost File411
$0.53 TELNET
$4.20 Estimated cost this search
$265.41 Estimated total session cost 4.587 DialUnits

SYSTEM:OS - DIALOG OneSearch
File 107:Adis R&D Insight 1986-2006/Sep W1
(c) 2006 Adis Data Information BV.
File 128:PHARMAPROJECTS 1980-2006/Nov W3
(c) 2006 Informa UK Ltd
*File 128: Please see HELP NEWS 128 for the latest
new information about the file.
File 340:CLAIMS(R)/US Patent 1950-06/Dec 05
(c) 2006 IFI/CLAIMS(R)
*File 340: The 2006 reload is online as of December 1, 2006.
IPCR/8 is available.
File 349:PCT FULLTEXT 1979-2006/UB=20061130UT=20061123
(c) 2006 WIPO/Thomson
*File 349: For important information about IPCR/8 and forthcoming
changes to the IC= index, see HELP NEWSIPCR.
File 357:Derwent Biotech Res. _1982-2006/Dec W2
(c) 2006 The Thomson Corp.
File 399:CA SEARCH(R) 1967-2006/UD=14524
(c) 2006 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
IPCR/8 classification codes now searchable as IC=. See HELP NEWSIPCR.
File 654:US Pat.Full. 1976-2006/Dec 05
(c) Format only 2006 Dialog
*File 654: IPCR/8 classification codes now searchable in 2006 records.
For information about IC= index changes, see HELP NEWSIPCR.

Set Items Description
--- ----- ----------
Executing TD343179174
Hilight option is not available in file(s) 399
HILIGHT set on as '%'
2644 GDF
19 MYO29
2 MYO029
0 MYO-29
2 MYO-029
S1 22 GDF AND (MYO29 OR MYO029 OR MYO-29 OR MYO-029)

>>>Duplicate detection is not supported for File 107.

>>>Duplicate detection is not supported for File 128.

>>>Duplicate detection is not supported for File 340.

>>>Duplicate detection is not supported for File 349.

>>>Duplicate detection is not supported for File 654.

>>>Records from unsupported files will be retained in the RD set.
S2 22 RD (unique items)
? t s2/7/1-22

2/7/1 (Item 1 from file: 107)
DIALOG(R)File 107:Adis R&D Insight
(c) 2006 Adis Data Information BV. All rts. reserv.

00234995 018197
DRUG NAME: Stamulumab
RECORD REVISION DATE: 20060627
SYNONYMS: Anti-%GDF%-8 antibody; Anti-myostatin antibody; MYO 029; MYO 29; %MYO-029%; Research programme: myostatin-blocking antibodies - Wyeth
CHEMICAL NAME: Immunoglobulin G1, anti-(human growth differentiation factor 8) (human MYO 029 heavy chain), disulfide wit h human MYO 029 lambda-chain, dimer
WHO ATC CODE: M09A - Other Drugs for Disorders of the Musculo-Skeletal System
EPHMRA ATC CODE: M5X - All Other Musculoskeletal Products
MECHANISM OF ACTION: Myostatin inhibitors; Transforming growth factor beta antagonists; Growth factor antagonists

ORIGINATOR COMPANY: Cambridge Antibody Technology (United Kingdom)
PARENT COMPANY: AstraZeneca
LICENSEE: Wyeth

HIGHEST PHASE: Phase II
DEVELOPMENT STATUS: Phase II, USA, Muscular dystrophy
Preclinical, USA, Muscle wasting

TEXT

Introduction:
Stamulumab (MYO 029, MYO 29, anti-myostatin antibody, anti-%GDF%-8 antibody) is a fully human IgG1 antibody product that inhibits myostatin (or growth differentiation factor-8; %GDF%-8). The antibody is under clinical development with Wyeth for potential use in muscle wasting disorders, including muscular dystrophy (MD) and age-related sarcopenia.
High expression levels of myostatin is associated with such muscle wasting disorders. Myostatin is a member of the TGF-beta (transforming growth factor-beta) family that functions as a negative regulator of skeletal muscle development and growth in mammals; this is essential to maintain normal muscle mass via prevention of excessive muscle growth. Stamulumab has been shown to suppress myostatin's activity in decreasing muscle mass, promoting fat accumulation and elevating blood glucose levels.
Company agreements
Stamulumab was discovered by Cambridge Antibody Technology (a subsidiary of AstraZeneca) in collaboration with Wyeth. Subsequently, Cambridge Antibody Technology licensed the antibody to Wyeth. In June 2006, Cambridge Antibody Technology was acquired by AstraZeneca/1/.
Key development milestones
Muscular dystrophy: Wyeth announced in February 2005 that a phase I/II trial has commenced among adult patients with MD in the US following successful IND filing. The prospective, randomised, placebo-controlled study is enrolling 108 patients at 12 clinical sites and involving equal numbers of patients with facioscapulohumeral MD (FSHD), Becker MD (BMD) and limb-girdle MD (LGMD). Trial findings are anticipated to become available in late 2006/2/ /3/.
Wyeth Research and the University of Pennsylvania School of Medicine have investigated the use of antibodies to block myostatin as potential treatment for diseases associated with muscle degeneration, such as MD. Initial preclinical studies of stamulumab in animal models of MD have shown promising effects.
Sarcopenia: Wyeth is also studying stamulumab as a potential therapy for age-related muscle wasting (sarcopenia); phase I trials were expected to begin in 2004/4/.

COMMERCIAL SUMMARY:
Muscle-wasting diseases, including muscular dystrophy and sarcopenia / Neutralises %GDF%-8 (myostatin)

Company	Region	Launch Date	Peak Sales	Patent Expiry
Cambridge Antibody Technology	Wrld	2011	$50m	
Cambridge Antibody Technology	Wrld	2011	$50m	

Muscular dystrophy / anti-myostatin antibody

Company	Region	Launch Date	Peak Sales	Patent Expiry

Wyeth Wrld 2012 $250m

PHARMACOLOGY OVERVIEW:
Antimicrobial activity:
Pharmacodynamics:
Increase muscle mass and reduces muscle degeneration in mice
Immunogenicity:
Mechanism of action:
Myostatin inhibitors
Transforming growth factor beta antagonists
Growth factor antagonists

CLINICAL OVERVIEW:
Route(s) of Administration: Injection
Administration Freq.(per day):
Drug Interactions:
Unknown.

Drug Interactions:

Pharmacodynamics (Musculoskeletal Disorders):
Preclinical studies: myostatin inhibition with stamulumab increased muscle mass by up to 20% in mice. In the mouse model of Duchenne muscular dystrophy, 4-week-old mdx mice were administered weekly intraperitoneal (IP) injections of stamulumab (60 mg/kg) or vehicle alone (control) for 3 months. At 3 months, treated mice had greater muscle size and strength with less evidence of muscle degeneration than control mice/5/.

2/7/2 (Item 1 from file: 128)
DIALOG(R)File 128:PHARMAPROJECTS

0025638
DRUG NAME: stamulumab
ORIGINATOR: Wyeth (USA) [Phase II Clinical Trial]
SYNONYMS: anti-%GDF%-8 MAb, Wyeth
anti-myostatin MAb, Wyeth
%MYO-029%
CAS REG NO: 705287-60-1
CHEM NAME: Immunoglobulin G1, anti-(human growth differentiation factor 8) (Human MYO-029 heavy chain, disulfide with human MYO-029 gam ma- chain, dimer
TEXT: Stamulumab (MYO-029) is an antimyostatin (anti-%GDF%-8) fully-human MAb, under development by Wyeth for the treatment of muscular dystrophy (MD) and age-related sarcopenia (wasting) (USAN Web Page, 8 Nov 2005). %GDF%-8 is associated with reduced skeletal muscle mass (Press releases, CAT, 8 Sep & 22 Nov 2004).

Marketing

The MAb was originally discovered in collaboration with Cambridge Antibody Technology (CAT) (AstraZeneca) and licensed to Wyeth (Press release, CAT, 8 Sep 2004). The MAb target came out of a project at MetaMorphix (Wyeth/Johns Hopkins University) to develop a series of growth and differentiation factors (%GDF%) for use in muscle wasting disorders, liver regeneration, autoimmune diseases and reproductive disorders. MetaMorphix sublicensed its Myostatin technology in 1999 to Wyeth for research and development in human therapeutics (Press release, MetaMorphix, 6 May 2002).

Clinical

Phase II

It is in a prospective, randomized, placebo-controlled US Phase I/ II trial in 108 adults with facioscapulohumeral, Becker or limb-girdle MD. Results are due in late 2006 (Press release, Wyeth, 23 Feb 2005; Interim Res, CAT, 16 May 2005).

Phase I

A Phase I trial in healthy adults was completed (Press release, CAT, 22 Nov 2004; Interim Res, CAT, 16 May 2005).

Preclinical

In mice, blockade of myostatin with MAbs increased muscle mass by up to 20% (R&D Day, Wyeth, 2 Jun 2004). An antimyostatin MAb given to mice over 3mth reduced the characteristics of Duchenne MD. %GDF%-9 (produced by ovaries and egg cells) and %GDF%-10 showed potential in the treatment of female infertility and pre-term labour, respectively. %GDF%-10 was being studied in foetal development and labour. %GDF%-12 had potential in liver repair and regeneration. %GDF%- 3 had potential in the treatment of autoimmune diseases and transplant rejection (Press releases, MetaMorphix, 6 May 2002 & Wyeth, 27 Nov 2002). Updated by LK on 10/11/2005.

STATUS: (Active)

World	Phase II Clinical Trial
Argentina	Available for Licensing
Australia	Available for Licensing
Austria	Available for Licensing
Belgium	Available for Licensing
Brazil	Available for Licensing
Canada	Available for Licensing
Chile	Available for Licensing
China	Available for Licensing
Colombia	Available for Licensing
Denmark	Available for Licensing
Finland	Available for Licensing
France	Available for Licensing
Germany	Available for Licensing
Greece	Available for Licensing
Hong Kong	Available for Licensing
India	Available for Licensing
Ireland	Available for Licensing
Israel	Available for Licensing
Italy	Available for Licensing
Japan	Available for Licensing
Luxembourg	Available for Licensing
Malaysia	Available for Licensing
Mexico	Available for Licensing
Netherlands	Available for Licensing
New Zealand	Available for Licensing
Norway	Available for Licensing
Peru	Available for Licensing
Philippines	Available for Licensing
Russian Federation	Available for Licensing
Portugal	Available for Licensing
South Africa	Available for Licensing
South Korea	Available for Licensing
Spain	Available for Licensing
Sweden	Available for Licensing
Switzerland	Available for Licensing
Thailand	Available for Licensing
Turkey	Available for Licensing
UK	Available for Licensing
USA	Phase II Clinical Trial/Available for Licensing
Venezuela	Available for Licensing

THER. CLASS: T3A2 (Monoclonal antibody, human)
M5Z (Musculoskeletal)
A14 (Anabolic)
ORIGIN: BI-P-A (Biological, protein, antibody)
RTE OF ADMIN:UN (Unknown)
INDICATIONS: Muscular dystrophy C2 Phase II Clinical Trial

Cachexia C1 Phase I Clinical Trial
PHARM. CODE: GF-%GDF%-8-AN Human growth and differentiation factor 8 antagonist Physiological, Hormonal, Human growth and differentiation factor 8 antagonist %GDF%-8 antagonist P-H-GF-%GDF%-8-AN

TARGET DATA CODE: 2660: GDF8; 2660: MSTN; 2660: myostatin

TARGET DATA:
2660: growth differentiation factor 8
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene&cmd=Retrieve&dopt=Graphics&list_uids=2660

	Therapy	Pharmacology	Status
LINKING:	T3A2	GF-%GDF%-8-AN	Phase II Clinical Trial
	M5Z	GF-%GDF%-8-AN	Phase II Clinical Trial
	A14	GF-%GDF%-8-AN	Phase I Clinical Trial

RATING: NOVELTY: 6 (Leading Compound)
DEVELOPMENT SPEED: 4 (Speed Rating - Faster than Average)
MARKET SIZE: 4 (Market Rating - US$ 5001-10000 million)
LATEST UPD: 20060630 (JB) Acquisition of Cambridge Antibody Technology by AstraZeneca reported
UPDATED: 20051108 (Est) Names Granted (MYO-029)
20050222 (Act) Change in Status (Phase I/II Clinical Trial)
20041122 (Est) Change in Status (Phase I Clinical Trial)
20030120 (Est) Compounds Identified (myostatin, MetaMorphix)
20020513 (Est) Development Continuing
19981215 (Est) No Development Reported
19970415 (Est) Licensing Opportunities (Worldwide)
19970415 (Est) New Product in Pharmaprojects
REVISED: 20060811

2/7/3 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent
(c) 2006 IFI/CLAIMS(R). All rts. reserv.

10776027 2005-0014733 2005-0003753
C/THERAPEUTIC AND PROPHYLACTIC METHODS FOR NEUROMUSCULAR DISORDERS
Document Type: Utility; Patent Application-First Publication
Inventors: Li Xiangping (US); Whittemore Lisa-Anne (US)
Assignee: Unassigned Or Assigned To Individual
Assignee Code: 68000
Probable Assignee (A1): Wyeth

Publication Number	Kind	Date	Application Number	Date
US 20050014733	A1	20050120	US 2004858353	20040601

Priority Applic: US 2004858353 20040601
Provisional Applic: US 60-474603 20030602

Abstract: The disclosure provides methods for treating neuromuscular disorders in mammals. The disclosed methods include administering therapeutically effective amounts of a %GDF%-8 inhibitor and a corticosteroid to a subject susceptible to, or having, a neuromuscular disorder, so as to maintain desirable levels of muscle function.

Exemplary Claim:
1. A method of treating a mammal with a decrease of muscle function, comprising administering to the mammal a therapeutically effective amount of at least one %GDF%-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat decrease of muscle function.

2/7/4 (Item 2 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent
(c) 2006 IFI/CLAIMS(R). All rts. reserv.

10635155 2004-0041473
C/NEUTRALIZING ANTIBODIES AGAINST %GDF%-8 AND USES THEREFOR; IMMUNOGLOBULIN
AGAINST GROWTH DIFFERENTIATION FACTOR -8 (%GDF%-8) FOR USE IN THE PREVENTION AND TREATMENT OF BONE, MUSCULAR, NERVOUS SYSTEM AND OBESITY;
MUSCLE ENHANCEMENT
Document Type: Utility; Patent Application-First Publication
Inventors: Bridges Kristie Grove (US); Davies Monique V (US); Field Anne (GB); Russell Caroline (GB); Song Kening (US); Valge-Archer Viia (GB); Veldman Geertruida M (US); Wolfman Neil M (US)
Assignee: Unassigned Or Assigned To Individual
Assignee Code: 68000
Probable Assignee (A1): Cambridge Antibody Technology Ltd GB; Wyeth

Publication Number	Kind	Date	Application Number	Date
US 20040142382	A1	20040722	US 2003688925	20031021

Priority Applic: US 2003688925 20031021
Provisional Applic: US 60-419964 20021022

Abstract: The disclosure provides novel antibodies against growth and differentiation factor-8 (%GDF%-8), in particular human antibodies, and antibody fragments, including those that inhibit %GDF%-8 activity in vitro and/or in vivo. The disclosure also provides methods for diagnosing, preventing, or treating degenerative disorders of muscle or bone, or disorders of insulin metabolism.

Exemplary Claim:
DRAWING

1. An isolated antibody comprising an amino acid sequence substantially as set out in SEQ ID. NO:n, wherein n is 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48; and wherein the antibody is capable of specifically binding %GDF%-8 or BMP-1.

2/7/5 (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT
(c) 2006 WIPO/Thomson. All rts. reserv.

01424634 **Image available**
DETECTION OF AN IMMUNE RESPONSE TO %GDF%-8 MODULATING AGENTS
DETECTION D'UNE REPONSE IMMUNITAIRE CONTRE LES AGENTS DE MODULATION %GDF%-8
Patent Applicant/Assignee:
WYETH, Five Giralda Farms, Madison, New Jersey 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
NOWAK John A, 11 William Circle, Stratham, New Hampshire 03885, US, US (Residence), US (Nationality), (Designated only for: US)
O'HARA Denise M, 8 Jere Road, Reading, Massachusetts 01867, US, US (Residence), GB (Nationality), (Designated only for: US)
CRYAN John G, 53 Hillando Drive, Shrewsbury, Massachusetts 01545, US, US (Residence), US (Nationality), (Designated only for: US)
CAIAZZO Teresa M, 160 Lancaster Drive, Tewksbury, Massachusetts 01876, US , US (Residence), US (Nationality), (Designated only for: US)
JOYCE Alison, 61 Cneter Street, Groveland, Massachusetts 01834, US, US (Residence), US (Nationality), (Designated only for: US)
RAJEWSKI Joseph W III, 34-36 H Street, Unit 2l, South Boston, Massachusetts 02127, US, US (Residence), US (Nationality), (Designated only for: US)
SUN Shujun, 43 Peabody Drive, Brentwood, New Hampshire 03833, US, US (Residence), US (Nationality), (Designated only for: US)

WOLFMAN Neil M, 5 Phillips Lane, Dover, Massachusetts 02030, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
FERGUSON Mary K (agent), FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER LLP, 901 New York Avenue, Washington, District Of Columbia 20001-4413, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 2006107611 A2 20061012 (WO 06107611)
Application: WO 2006US10711 20060323 (PCT/WO US2006010711)
Priority Application: US 2005664643 20050323
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KN KP KR KZ LC LK LR LS LT LU LV LY MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU LV MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Main International Patent Class (v7): none
Publication Language: English
Filing Language: English
Fulltext Word Count: 21928

English Abstract
This disclosure provides methods for the detection of antibodies to a %GDF%-8 modulating agent such as, e.g., MYO-029, in a biological sample. Methods to detect an immune response to a %GDF%-8 modulating agent are also included. In particular, methods to assess an immune response in animals, including humans, to a %GDF%-8 modulating agent such as a %GDF%-8 inhibitor are provided herein.

French Abstract
L'invention concerne des procedes de detection d'anticorps contre un agent de modulation %GDF%-8, par exemple, le MYO-029, dans un prelevement biologique. De plus, l'invention concerne des procedes de detection d'une reponse immunitaire contre un agent de modulation %GDF%-8. En particulier, l'invention concerne des procedes d'evaluation d'une reponse immunitaire chez les animaux, y compris les humains, contre un agent de modulation %GDF%-8, tel que l'inhibiteur %GDF%-8.

Legal Status (Type, Date, Text)
Publication 20061012 A2 Without international search report and to be republished upon receipt of that report.

Claim
CLAIMS

1. A method to detect an antibody that specifically binds to a %GDF%-8 modulating agent in a biological sample, comprising: (a) adding the %GDF%-8 modulating agent to an in vitro assay for a %GDF%-8 activity in a reaction vessel; (b) adding the biological sample to the in vitro assay for a %GDF%-8 activity in the reaction vessel; (c) detecting modulation of the %GDF%-8 activity by the biological sample; and (d) comparing the modulation of the %GDF%-8 activity in the presence of the biological sample to the modulation of the %GDF%-8 activity in the presence of the %GDF%-8 modulating agent alone.

2. The method of claim 1, wherein the in vitro assay is an immunoassay comprising: (a) contacting the %GDF%-8 modulating agent with a surface of the reaction vessel; (b) subsequently adding the biological sample to the reaction vessel; (c) adding a detection agent to the reaction vessel; and (d) detecting a %GDF%-8 modulating agent/antibody complex associated with the surface.

3. The method of claim 2, wherein the detection agent is the %GDF%-8 modulating agent with a detectable label.

4. The method of claim 2, wherein the detection agent is a labeled %GDF%-8 protein.

5. The method of claim 4, wherein the label is biotin.

6. The method of claim 5, wherein the ratio of moles of biotin incorportated to moles of agent is less than 5:1.

7. The method of claim 5, wherein the ratio of biotin to agent is between about 0.5:1 to 4:1.

8. A method to detect an antibody that specifically binds to a %GDF%-8 modulating agent in a biological sample, comprising: (a) contacting the %GDF%-8 modulating agent with a surface of a reaction vessel; (b) adding the biological sample to the reaction vessel;.
(c) adding a detection agent to the reaction vessel; and (d) detecting a %GDF%-8 modulating agent/antibody complex associated with the surface of the reaction vessel.

9. The method of claim 8, wherein the detection agent is the %GDF%-8 modulating agent of step (a) with a detectable label.

10. The method of claim 8, wherein the detection agent is a labeled %GDF%-8 protein.

11. The method of claim 10, wherein the %GDF%-8 modulating agent/antibody complex is detected by comparing %GDF%-8 modulating agent/labeled %GDF%-8 protein complex levels in the test sample to levels in a control sample.

12. The method of claim 8, wherein the %GDF%-8 modulating agent is a %GDF%-8 inhibitor.

13. The method of claim 12, wherein the %GDF%-8 inhibitor is an antibody.

14. The method of claim 13, wherein the antibody specifically binds to %GDF%-8.
15. The method of claim 14, wherein the antibody is MYO-029.

16. The method of claim 8, wherein the %GDF%-8 modulating agent is chosen from: (a) an antibody that specifically binds to %GDF%-8; (b) an antibody that specifically binds to a %GDF%-8 binding partner; (c) a soluble %GDF%-8 receptor; (d) an ActRIIB protein; (e) a follistatin-domain containing protein; (f) a follistatin protein; (g) a GASP-I protein; (h) a %GDF%-8 protein; (I) a %GDF%-8 propeptide; (j) a non-proteinacious inhibitor; (k) a nucleic acid; and (l) a small molecule.

17. The method of claim 8, wherein the biological sample is from a mammal, bird, reptile, or fish.

18. The method of claim 17, wherein the biological sample is from a mammal.

19. The method of claim 18, wherein the mammal is a human.

20. The method of claim 8, wherein the biological sample is chosen from serum, blood, plasma, biopsy sample, tissue sample, cell suspension, saliva, oral fluid, cerebrospinal fluid, amniotic fluid, milk, colostrum, mammary gland secretion, lymph, urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, and mucus.

21. The method of claim 20, wherein the biological sample is chosen from serum, blood, and plasma.

22. The method of claim 10, wherein the label is chosen from an enzyme, an epitope tag, a radiolabel, biotin, a dye, a fluorescent tag label, and a luminescent label.

23. The method of claim 22, wherein the label is biotin.

24. The method of claim 23, wherein the ratio of moles of biotin incorportated to moles of detection agent is less than 5:1.

25. The method of claim 23, wherein the ratio of biotin to agent is between about 0.5:1 to 4:1.

26. The method of claim 23, further comprising adding an avidin-enzyme conjugate. S 27. The method of claim 26, further comprising adding a substrate that changes color, luminescence, or fluorescence in the presence of the enzyme.
28. A method to detect an antibody that specifically binds to a %GDF%-8 inhibitor in a biological sample, comprising: (a) contacting a first %GDF%-8 inhibitor with a surface of a reaction vessel; (b) adding the biological sample to the reaction vessel; (c) adding a labeled second %GDF%-8 inhibitor to the reaction vessel; and (d) detecting the labeled second %GDF%-8 inhibitor associated with the surface.

29. The method of claim 28, wherein the biological sample is from a mammal, bird, reptile, or fish.

30. The method of claim 29, wherein the biological sample is from a mammal.

31. The method of claim 30, wherein the mammal is a human.

32. The method of claim 28, wherein the biological sample is chosen from serum, blood, plasma, biopsy sample, tissue sample, cell suspension, saliva, oral fluid, cerebrospinal fluid, amniotic fluid, milk, colostrum, mammary gland secretion, lymph, urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, and mucus.

33. The method of claim 32, wherein the biological sample is chosen from serum, blood, and plasma.

34. The method of claim 28, wherein the first %GDF%-8 inhibitor and the second %GDF%-8 inhibitor are the same.

35. The method of claim 28, wherein the first %GDF%-8 inhibitor is an antibody that specifically binds to %GDF%-8.

36. The method of claim 28, wherein the second %GDF%-8 inhibitor is an antibody that specifically binds to %GDF%-8.

37. The method of claim 28, wherein the label is chosen from an enzyme, an epitope tag, a radiolabel, biotin, a dye, a fluorescent tag label, and a luminescent label.

38. The method of claim 28, wherein the label is biotin.

39. The method of claim 38, further comprising adding an avidin-enzyme conjugate.

40. The method of claim 39, further comprising adding a substrate that changes color, luminescence, or fluorescence in the presence of the enzyme.

41. A method to detect an antibody that specifically binds to MYO-029 in a biological sample, comprising: (a) contacting isolated MYO-029 with a surface of a reaction vessel; (b) adding the biological sample to the reaction vessel; (c) adding labeled MYO-029 to the reaction vessel; and (d) detecting labeled MYO-029 associated with the surface.

42. A method to detect an antibody that specifically binds to MYO-029 in a biological sample, comprising: (a) providing a host cell comprising a reporter gene construct in a reaction vessel, wherein the construct comprises a %GDF%-8-responsive control element and a reporter gene; (b) adding an amount of mature %GDF%-8 protein to the vessel sufficient to activate expression of the reporter gene; (c) adding an amount of MYO-029 to the vessel of step (b) sufficient to modulate the %GDF%-8 activation of the reporter gene; (d) adding a biological sample to the reaction vessel of step (c); and (e) detecting reporter gene expression in the presence and absence of the biological sample, 43. The method of claim 41, wherein the biological sample is from a mammal, bird, reptile, or fish.

44. The method of claim 43, wherein the biological sample is from a mammal.

45. The method of claim 44, wherein the mammal is a human.

46. The method of claim 41, wherein the biological sample is chosen from serum, blood, plasma, biopsy sample, tissue sample, cell suspension, saliva, oral fluid, cerebrospinal fluid, amniotic fluid, milk, colostrum, mammary gland secretion, lymph,urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, and mucus.

47. The method of c'aim 46, wherein the biological sample is chosen from serum, blood, and plasma.

48. The method of claim 41, wherein the label is chosen from an enzyme, an epitope tag, a radiolabel, biotin, a dye, a fluorescent tag label, and a luminescent label.

49. The method of claim 48, wherein the label is biotin.

50. The method of claim 49, wherein the median ratio of moles of biotin incorporated to moles of agent is at least 5:1.
51. The method of claim 49, wherein the median ratio of biotin to agent is at least 10:1.

52. The method of claim 49, further comprising adding an avidin-enzyme conjugate.

53. The method of claim 52, further comprising adding a substrate that changes color, luminescence, or fluorescence in the presence of the enzyme.

54. A method to detect an antibody that specifically binds to MYO-029 in a biological sample, comprising: (a) contacting isolated MYO-029 with a surface of a reaction vessel; (b) adding the biological sample to' the reaction vessel; (c) adding labeled %GDF%-8 to the reaction vessel; and (d) detecting labeled %GDF%-8 associated with the surface in the presence and absence of the biologlcaf sample.

55. The method of claim 54, wherein the biological sample is from a mammal, bird, reptile, or fish.

56. The method of claim 55, wherein the biological sample is from a mammal.
57. The method of claim 56, wherein the mammal is a human.

58. The method of claim 54, wherein the biological sample is chosen from serum, blood, plasma, biopsy sample, tissue sample, cell suspension, saliva, oral fluid, cerebrospinal fluid, amniotic fluid, milk, colostrum, mammary gland secretion, lymph, urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, and mucus. " 59. The method of claim 54, wherein the biological sample is chosen from serum, blood, and plasma.

60. The method of claim 54, wherein the label is chosen from an enzyme, an epitope tag, a radiolabel, biotin, a dye, a fluorescent tag label, and a luminescent label.

61. The method of claim 60, wherein the label is biotin.

62. The method of claim 61, further comprising adding an avidin-enzyme conjugate.

63. The method of claim 62, further comprising adding a substrate that

changes color, luminescence, or fluorescence in the presence of the enzyme.

64. A method to assess an individual's immune response to a first %GDF%-8 inhibitor, the method comprising: (a) contacting a first %GDF%-8. inhibitor with a surface of a reaction vessel; (b) adding a biological sample from an individual to the reaction vessel; (c) adding a labeled second %GDF%-8 inhibitor to the reaction vessel; and (d) detecting a labeled second %GDF%-8 inhibitor/antibody complex associated with the surface, wherein detection of labeled complex indicates an immune response to the first %GDF%-8 inhibitor.

65. A method to assess an individual's immune response to a first %GDF%-8 inhibitor, the method comprising (a) contacting a %GDF%-8 inhibitor with a surface of a reaction vessel; (b) adding a biological sample from an individual to the reaction vessel; (c) adding a labeled %GDF%-8 protein to the reaction vessel; and (d) comparing the amount of labeled %GDF%-8 protein associated with the surface in the test sample to a control sample, wherein detection of a decreased level of labeled complex indicates an immune response to the %GDF%-8 inhibitor.

66. A method to assess an individual's immune response to a first %GDF%-8 inhibitor, the method comprising: (a) providing a host cell comprising a reporter gene construct in a reaction vessel, wherein the construct comprises a %GDF%-8-responsive control element and a reporter gene; (b) adding an amount of mature %GDF%-8 protein to the vessel sufficient to activate expression of the reporter gene; (c) adding an amount of MYO-029 to the vessel of step (b) sufficient to modulate the %GDF%-8 activation of the reporter gene; (d) adding a biological sample to the reaction vessel of step (c); and (e) detecting reporter gene expression in the presence and absence of the biological sample.

2/7/6 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01344397
PRODUCTION OF POLYPEPTIDES
PRODUCTION DE POLYPEPTIDES
Patent Applicant/Assignee:
WYETH RESEARCH IRELAND LIMITED, Little Connell, Newbridge, County Kildare , IE, IE (Residence), IE (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
DRAPEAU Denis, 55 Old Farm Road, Salem, NH 03079, US, US (Residence), US (Nationality), (Designated only for: US)
LUAN Yen-Tuang, 3 Armand Drive, Chelmsford, MA 01824, US, US (Residence), US (Nationality), (Designated only for: US)
MERCER James R, 226 Hampstead Road, Derry, NH 03038, US, US (Residence), US (Nationality), (Designated only for: US)
WANG Wenge, 1 Hollowridge Road, North Chelmsford, MA 01863, US, US (Residence), CN (Nationality), (Designated only for: US)
LASKO Daniel, 19 Gleason Street, Medford, MA 02155, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
JARRELL Brenda Herschbach et al (agent), Choate, Hall & Stewart LLP, Two International Place, Boston, MA 02110, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 200626445 A1 20060309 (WO 0626445)
Application: WO 2005US30437 20050826 (PCT/WO US2005030437)
Priority Application: US 2004605097 20040827; US 2004604941 20040827; US 2004605074 20040827
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU LV MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM

International Patent Class (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
C12P-0021/02 A I F B 20060101 H EP
C07K-0016/00 A I L B 20060101 H EP
C12N-0005/00 A I L B 20060101 H EP
C07K-0016/22 A I L B 20060101 H EP
C07K-0016/30 A I L B 20060101 H EP
Publication Language: English
Filing Language: English
Fulltext Word Count: 35678

English Abstract
An improved system for large scale production of proteins and/or polypeptides in cell culture, particularly in media characterized by one or more of: i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of it maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease

French Abstract
L'invention porte sur un systeme ameliore de production a grande echelle de proteines et/ou de polypeptides dans une culture cellulaire, notamment dans des milieux caracterises par au moins: i) une concentration d'acides amines cumules superieure a environ 70 mM; ii) un rapport molaire de glutamine cumulee/asparagine cumulee inferieur a environ 2; iii) un rapport molaire de glutamine cumulee/acides amines cumules inferieur a environ 0.2; iv) un rapport molaire d'ions inorganiques cumules/acides amines cumules compris entre environ 0,4 et 1; ou v) une concentration combinee de glutamine cumulee et d'asparagine cumulee comprise entre environ 16 et 36 mM. L'utilisation de ce systeme permet de parvenir a des niveaux eleves de production de proteines et de reduire l'accumulation de certains facteurs indesirables tels que l'ammonium et/ou le lactate. De plus, les procedes de mise en culture presentent un ecart de temperature tel que, generalement, une baisse de la temperature lorsque la culture a atteint environ 20 a 80 % de sa densite cellulaire maximale. En variante ou de plus, cette invention porte sur des procedes dans lesquels, apres avoir atteint une crete, les taux de lactate et/ou d'ammonium de la culture diminuent au fil du temps.

Legal Status (Type, Date, Text)
Publication 20060309 A1 With international search report.
Publication 20060309 A1 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Claim
I . A method of producing a polypeptide in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a medium characteristic selected from the group consisting of- (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative

glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM,
and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

2 A method of producing a polypeptide in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and
said medium containing glutamine; and
84
said medium having two medium characteristics selected from the group consisting of. (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to
cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per
unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

3 A method of producing a polypeptide in a large-scale production cell culture
comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of- (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per
unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a
85
viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

4 A method of producing a polypeptide in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of- (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (H) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv)
a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
86
. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of
providing a cell Culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutainine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of
greater than about 16 mM, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

6 A method of producing a polypeptide in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and
said medium containing glutamine; and
87

said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between
about 0.4 to 1, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
7 The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of- (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

8 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 13 niM.

9 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 10 mM.

10 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 niM.

11 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.
88
. The method of claim 1, wher ein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.

13 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.
14 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.

15 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.

16 The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.

17 The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 pN4.

18 The method of claim 1, wherein viable cell density of said culture is measured on a periodic basis.

19 The method of claim 1, wherein viability of said culture is measured on a periodic basis.

20 The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.

21 The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.

22 The method of claim 1, wherein said titer of said culture is measured on a periodic basis.
89
. The method of claim 1, wherein osmolarity of said culture is measured on a periodic basis.

24 The method of claims 18-23, wherein said measurements are taken daily.

25 The method of claim 1, wherein the initial density of said mammalian cells is at least 2xl 02 cells/mL.

26 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x l03 cells/mL.

27 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x IO 4cells/mL.

28 The method of claim 1, wherein the initial density of said mammalian cells is at least 2xlO5 cells/mL.
29 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x l06 cells/mL.

30 The method of claim 1, wherein the initial density of said mammalian cells is at least 5xl 06 cells/mL.

31 The method of claim 1, wherein the initial density of said mammalian cells is at least IOXI06 cells/mL.

32 The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.

33 The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.

34 The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.
90
. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.

36 The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.

37 The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.

38 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.

39 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.

40 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.

41 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.

42 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.

43 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.

44 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.

45 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.
91
. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 33 degrees Celsius.

47 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.

48 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.

49 The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.

50 The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of. (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

51 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40 degrees Celsius.

52 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.

53 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.

54 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.

55 The method of claim 1, wherein said first period of time is between 0-8 days.

56 The method of claim 1, wherein said first period of time is between 1-7 days.

92. The method of claim 1, wherein said first period of time is between 2-6 days.

58 The method of claim 1, wherein said first period of time is between 3-5 days.

59 The method of claim 1, wherein said first period of time is approximately 4 days.

60 The method of claim 1, wherein said first period of time is approximately 5 days.

61 The method of claim 1, wherein said first period of time is approximately 6 days.

62 The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.

63 The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.

64 The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.

65 The method of claim 1, wherein said total amount of said produced polypeptide is at least 1 fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

66 The method of claim 1, wherein said total amount of said produced polypeptide is at least 2-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

93. The method of claim 1, wherein said cell culture is further provided with supplementary components.

68 The method of claim 67, wherein said supplementary components are provided at multiple intervals.

69 The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nueleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.

70 A method of producing a polypeptide in a large-scale production cell culture
comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of. i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

94. A method of producing a polypeptide in a large-scale production cell culture
comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of. i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutainine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

72 A method of producing a polypeptide in a large-scale production cell culture
comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and

a defined medium containing glutainine and having at least four medium characteristic selected from the group consisting of- i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM; maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a
95
range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

73 A method of producing a polypeptide in a large-scale production cell culture
comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, H) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about
mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
74 A method of producing a polypeptide in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
96
a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that the polypeptide accumulates in the cell culture.

75 The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of(i) a starting amino acid concentration greater than about 70 mM, (ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.

76 The method of any one of claims 1-6 or 70-75, wherein:
lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;
ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and
total amount of produced polypeptide is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

77 The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said polypeptide.
97
. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said polypeptide.

79 The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.

80 The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.

81 The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.

82 The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.
83 The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.

84 The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylaianine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
98
. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 3 5 mM.

86 The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
87 The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

88 The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of

(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;
(v) a cumulative total amount of phenylalanine per unit volume greater than
approximately 2.5 mM;
(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;
(vii) a cumulative total amount of tryptophan per unit volume greater than
approximately 1.0 mM;
(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.
99
. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of(i) an initial amount of histidine per unit volume greater than approximately
1.7 mM;
(ii) an initial amount of isoleucine per unit volume greater than approximately
3.5 mM;
(iii) an initial amount of leucine per unit volume greater than approximately
5.5 mm;
(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;
(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) an initial amount of proline per unit volume reater than approximately 2.5
mm;
(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) an initial amount of tyrosine per unit volume greater than approximately
2.0 mM; and
(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.

90 The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.

91 The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately IO mM.

92 The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

93 The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
100
. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

95 The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.

96 The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.

97 The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.

98 The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately I mM.

99 The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L.
100. Themethodofclaiml,whereinthecumulativetotalamountofcalcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L.
101. Themethodofclaiml,whereinthecumulativetotalamountofnicotinamide per unit volume in said medium is greater than approximately 7 mg/L. 102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L. 103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mg/L. 104. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.
101
. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L. 106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L.
107. Themethodofclaimlwhereinthecumulativetotalamountofthiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L. 108. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 3 5 mg/L.
109. Themethodofanyofelaimsl-23orclaims25-75orclaims77-108,wherein the polypeptide is anti-%GDF%
110. Themethodofanyofelaimsl-23orelaims25-75orelaims77-108,wherein the polypeptide is anti-LewY.
I 1 1. The method of claim 24, wherein the polypeptide is anti-%GDF%
112. The method of claim 24, wherein the polypeptide is anti-LewY. 113. Themethodofclaim76,whereinthepolypeptideisanti-%GDF%-S. 114. The method of claim 76, wherein the polypeptide is anti-LewY.
102

2/7/7 (Item 3 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01343461 **Image available**
PRODUCTION OF TNFR-Ig FUSION PROTEIN
PRODUCTION DE TNFR-IG
Patent Applicant/Assignee:
WYETH RESEARCH IRELAND LIMITED, Little Connell, Newbridge, County Kildare , IE, IE (Residence), IE (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
DRAPEAU Denis, 55 Old Farm Road, Salem, NH 03079, US, US (Residence), US (Nationality), (Designated only for: US)
LUAN Yen-Tuang, 3 Armand Drive, Chelmsford, MA 01824, US, US (Residence), US (Nationality), (Designated only for: US)
MERCER James R, 226 Hampstead Road, Derry, NH 03038, US, US (Residence), US (Nationality), (Designated only for: US)
WANG Wenge, 1 Hollowridge Road, North Chelmsford, MA 01863, US, US (Residence), CN (Nationality), (Designated only for: US)
LASKO Daniel, 19 Gleason Street, Medford, MA 02155, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
JARRELL Brenda Herschbach (agent), Choate, Hall & Stewart LLP, Two International Place, Boston, MA 02110, US

Patent and Priority Information (Country, Number, Date):
Patent: WO 200626447 A2-A3 20060309 (WO 0626447)
Application: WO 2005US30439 20050826 (PCT/WO US2005030439)
Priority Application: US 2004605379 20040827
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU LV MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM

International Patent Class (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
C12P-0021/02 A I F B 20060101 H EP
C12N-0015/62 A I L B 20060101 H EP
Publication Language: English
Filing Language: English
Fulltext Word Count: 35615

English Abstract
An improved system for large scale production of a dimeric fusion protein consisting of the extracellular ligang-binding portion of the human (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of IgGI in cell culture, particularly in media characterized by one or more of: i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of it maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time.

French Abstract
La presente invention a trait a un systeme ameliore pour la production sur une grande echelle de proteines et/ou de polypeptides en culture cellulaire, notamment dans des milieux caracterises par un ou plusieurs des elements suivants: i) une concentration cumulative d'acides amines superieure a environ 70 mM; ii) un rapport glutamine cumulative molaire/asparagine cumulative inferieur a environ 2; iii) un rapport glutamine cumulative/total acides amines cumulatif inferieur a 0,2; iv) un rapport ion inorganique cumulatif molaire/total acides amines entre environ 0,4 et 1; ou v) une concentration combinee de glutamine cumulative et d'asparagine cumulative entre environ 16 et 36 mM. L'utilisation d'un tel systeme permet des niveaux eleves de production de proteines et reduit l'accumulation de certains facteurs indesirables tels que l'ammonium et/ou le lactate. La presente invention a egalement trait a des procedes de culture comprenant un decalage de la temperature, typiquement une baisse dans la temperature lorsque la culture a atteint environ 20-80 % de sa densite cellulaire maximale. En variante ou en outre, la presente invention a trait a des procedes tels, qu'apres avoir atteint un pic, les niveaux d'ammonium et/ou de lactate dans la culture diminue dans le temps.

Legal Status (Type, Date, Text)
Publication 20060309 A2 Without international search report and to be republished upon receipt of that report.
Search Rpt 20060420 Late publication of international search report
Republication 20060420 A3 With international search report.
Republication 20060420 A3 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.
Search Rpt 20060420 Late publication of international search report
Correction 20060601 Corrected version of Pamphlet: pages 1/38-38/38, drawings, replaced by new pages 1/38-38/38
Republication 20060601 A3 With international search report.

Claim
I . A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a medium characteristic selected from the group consisting of. (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative tota! amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

2 A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and
said medium containing glutamine; and
84
said medium having two medium characteristics selected from the group consisting of. (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to
cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per
unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

3 A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and

a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per
unit volume of greater than about 16 mM, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a
85
viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

4 A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutarnine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume
of greater than about 16 mM, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.
86
. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of
greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

6 A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and
said medium containing glutamine; and
87
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between
about 0.4 to 1, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

7 The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of. (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

8 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 13 mM.

9 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to IO mM.

10 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 mM. IL The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.
88
. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.
13 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to IO mM.

14 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.

15 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.

16 The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.

17 The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 gM.

18 The method of claim 1, wherein viable cell density of said culture is

measured on a periodic basis.

19 The method of claim 1, wherein viability of said culture is measured on a periodic basis.

20 The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.

21 The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.

22 The method of claim 1, wherein said titer of said culture is measured on a periodic basis.
89
. The method of claim 1, wherein osmolarity of said culture is measured on a periodic basis.

24 The method of claims 18-23, wherein said measurements are taken daily.

25 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x I02 cells/mL.

26 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x I03 cells/mL.

27 The method of claim 1, wherein the initial density of said mammalian cells is
' 104
at least 2x cells/mL.

28 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x I05 cells/mL.

29 The method of claim 1, wherein the initial density of said mammalian cells is at least 2x 106 cells/mL.

30 The method of claim 1, wherein the initial density of said mammalian cells is at least 5xI 06 cells/mL.

31 The method of claim 1, wherein the initial density of said mammalian cells is at least IOXI06 cells/mL.

32 The method of claim 1, wherein the step of providing comprises providing at least about I 000 L of a culture.

33 The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.

34 The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.
90
. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.

36 The method of claim 1, wherein the step of providing comprises providing at least about I 0,000 L of a culture.

37 The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.

38 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.

39 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.

40 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.

41 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.
42 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.

43 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.

44 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.

45 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.
91
. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 33 degrees Celsius.

47 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.
48 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.

49 The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.

50 The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of. (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

51 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40 degrees Celsius.

52 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.

53 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.
54 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.

55 The method of claim 1, wherein said first period of time is between 0-8 days.

56 The method of claim 1, wherein said first period of time is between 1-7 days.
92
. The method of claim 1, wherein said first period of time is between 2-6 days.

58 The method of claim 1, wherein said first period of time is between 3-5 days.

59 The method of claim 1, wherein said first period of time is approximately 4 days.

60 The method of claim 1, wherein said first period of time is approximately 5 days.

61 The method of claim 1, wherein said first period of time is approximately 6 days.

62 The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.

63 The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.

64 The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.

65 The method of claim 1, wherein said total amount of said produced TNFR-Ig is at least 1 fold higher that the amount of TNFR-Ig produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

66 The method of claim 1, wherein said total amount of said produced TNFR-Ig is at least 2-fold higher that the amount of TNFR-Ig produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

93
. The method of claim 1, wherein said cell culture is further provided with supplementary components.

68 The method of claim 67, wherein said supplementary components are provided at multiple intervals.

69 The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.

70 A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of. i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell
culture,

94
. A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 2.0%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

72 A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of,
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least four medium characteristic selected from the group consisting of. i) a starting amino acid concentration greater than about 70 mM, H) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a
95
range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

73 A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about
16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

74 A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-1g, which gene is expressed under condition of cell culture; and
96
a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow

said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that TNFR-Ig accumulates in the cell culture.
75 The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of(i) a starting amino acid concentration greater than about 70 mM, (ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.

76 The method of any one of claims 1-6 or 70-75, wherein:
lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;
ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and
total amount of produced TNFR-Ig is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

77 The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said TNFR-1g.
97
. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said TNFR-Ig.

79 The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.

80 The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.

81 The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.

82 The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.

83 The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.

84 The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
98
. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

86 The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.

87 The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

88 The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of.
(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;
(v) a cumulative total amount of phenylalanine per unit volume greater than
approximately 2.5 mM;
(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;
(vii) a cumulative total amount of tryptophan per unit volume greater than
approximately 1.0 mM;
(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.
99
. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of. (i) an initial amount of histidine per unit volume greater than approximately
1.7 mM;
(ii) an initial amount of isoleucine per unit volume greater than approximately
3.5 mM;
(iii) an initial amount of leucine per unit volume greater than approximately
5.5 mM;
(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;
(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) an initial amount of proline per unit volume reater than approximately 2.5
mm;
(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) an initial amount of tyrosine per unit volume greater than approximately
2.0 mM; and
(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.

90 The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.

91 The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately IO mM.

92 The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8

mM.
93 The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
100
. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

95 The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.

96 The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.

97 The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.

98 The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately I mM.

99 The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L. 100. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L. 101. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 7 mg/L. 102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L. 103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mg/L. 104. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.
101
. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L. 106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L. . 107. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L. 108. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.
102

2/7/8 (Item 4 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01342277 **Image available**
PRODUCTION OF ANTI-AMYLOID BETA ANTIBODIES
PRODUCTION D'$G(A)-ABETA
Patent Applicant/Assignee:
WYETH RESEARCH IRELAND LIMITED, Little Connell, Newbridge, County Kildare , IE, IE (Residence), IE (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
DRAPEAU Denis, 55 Old Farm Road, Salem, NH 03079, US, US (Residence), US (Nationality), (Designated only for: US)
LUAN Yen-Tuang, 3 Armand Drive, Chelmsford, MA 01824, US, US (Residence), US (Nationality), (Designated only for: US)
MERCER James R, 226 Hampstead Road, Derry, NH 03038, US, US (Residence), US (Nationality), (Designated only for: US)
WANG Wenge, 1 Hollowridge Road, North Chelmsford, MA 01863, US, US (Residence), CN (Nationality), (Designated only for: US)
LASKO Daniel, 19 Gleason Street, Medford, MA 02155, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
JARRELL Brenda Herschbach (agent), Choate, Hall & Stewart LLP, Two International Place, Boston, MA 02110, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 200626408 A2-A3 20060309 (WO 0626408)
Application: WO 2005US30364 20050826 (PCT/WO US2005030364)
Priority Application: US 2004604936 20040827
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU LV MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM

International Patent Class (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
C12P-0021/08 A I F B 20060101 H EP
Publication Language: English
Filing Language: English
Fulltext Word Count: 35622

English Abstract
An improved system for large scale production of anti-amyloid beta antibodies in cell culture, particularly in media characterized by one or more of i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of its maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time.

French Abstract
Cette invention concerne un systeme ameliore de production a grande echelle de proteines et/ou de polypeptides en culture cellulaire, en particulier dans un milieu caracterise par un ou plusieurs des elements suivants: i) une concentration d'acides amines cumules superieure a environ 70 mM; ii) un rapport molaire glutamine cumulee/asparagine cumulee inferieur a environ 2; iii) un rapport molaire glutamine cumulee/total d'acides amines cumules inferieur a environ 0,2; iv) un rapport molaire ion inorganique cumule/total d'acides amines cumules compris entre environ 0,4 et 1; et v) une concentration combinee de glutamine cumulee et d'asparagine cumulee comprise entre environ 16 et 36 mM. L'utilisation d'un tel systeme permet de produire des proteines en grandes quantites et de reduire l'accumulation de certains facteurs indesirables tels que l'ammonium et/ou le lactate. Cette invention concerne egalement des procedes de culture comprenant un ecart de temperature, habituellement une baisse de temperature lorsque la culture a atteint entre 20 et 80 % de sa densite cellulaire maximale. Dans une variante ou en outre, cette invention concerne des procedes selon lesquels les niveaux de lactate et/ou d'ammonium, apres avoir atteint un sommet, diminuent progressivement dans la culture.

Legal Status (Type, Date, Text)
Publication 20060309 A2 Without international search report and to be republished upon receipt of that report.

Search Rpt 20060504 Late publication of international search report
Republication 20060504 A3 With international search report.
Republication 20060504 A3 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Claim

1 . A method of producing a-ABeta in a large-scale production cell culture
comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a medium characteristic selected from the group consisting of. (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (H) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

2 A method of producing a-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and
said medium containing glutamine; and
84
sai'd niedium having two medium characteristics selected from the group consisting of. (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to
cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per
unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

3 A method of producing a-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per
unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a
85
viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

4 A method of producing a-ABeta in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv)
a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.
86
.... - @ A"ffiRfi6d 6. f "producing a-ABeta in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of
greater than about 16 mM, and combinations thereof,
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture

conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

6 A method of producing a-ABeta in a large-scale production cell culture comprising the steps of
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and
said medium containing glutamine; and
87
said medium having two medium characteristics selected from the group consisting of. (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between
about 0.4 to 1, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions; changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

7 The method of claim I, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

8 The method of claim 1. wherein the initial glutamine concentration of said medium is less than or equal to 13 mM.

9 The method of claim 1. wherein the initial glutamine concentration of said medium is less than or equal to IO mM.

10 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 mM.

11 The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.
88
. The method ot'claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.

13 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.

14 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.

15 The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.

16 The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.

17 The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 gM. I 8. The method of claim 1, wherein viable cell density of said culture is measured on a periodic basis.

19 The method of claim 1, wherein viability of said culture is measured on a periodic basis.

20 The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.

21 The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.

22 The method of claim 1, wherein said titer of said culture is measured on a periodic basis.
89
'21 ..'The m- Rji6d 6--f claim 1, wherein osmolarity of said culture is measured on a periodic basis.

24 The method of claims 18-23, wherein said measurements are taken daily.

25 The method of claim 1, wherein the initial density of said mammalian cells is at least 2xl 02 cells/mL.

26 The method of claim 1, wherein the initial density of said mammalian cells is at least 2xl 03 cells/mL.

27 The method of claim 1, wherein the initial density of said mammalian cells is at least 2xl 04 cells/mL.

28 The method of claim I. wherein the initial density of said mammalian cells is at least 2xlO5 cells/mL.

29 The method of claim 1, wherein the initial density of said mammalian cells is at least 2xl 06 cells/mL.
30 The method of claim 1, wherein the initial density of said mammalian cells is at least 5xl 06 cells/mL.

31 The method of claim 1, wherein the initial density of said mammalian cells is at least IOXI06 cells/mL.

32 The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.

33 The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.

34 The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.
90
. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.

36 The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.

37 The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.

38 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.

39 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.

40 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.

41 The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.

42 The method of claim 1. wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.

43 The method of claim 1, wherein said second set of conditions comprises

a second temperature range that is approximately 25 to 41 degrees Celsius.

44 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.

45 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.
91
i'MX
in df claim 1, wherein said second set of conditions comprises a
. ..... .TIW dtho'8
second temperature range that is approximately 29 to 33 degrees Celsius.

47 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.

48 The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.

49 The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.

50 The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of- (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.

51 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40 degrees Celsius.

52 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.

53 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.

54 The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.

55 The method of claim 1, wherein said first period of time is between 0-8 days.
56 The method of claim 1, wherein said first period of time is between 1-7 days.
92
. The method of claim 1, wherein said first period of time is between 2-6 days.

58 The method of claim 1. wherein said first period of time is between 3-5 days.

59 The method of claim 1, wherein said first period of time is approximately 4 days.

60 The method of claim 1, wherein said first period of time is approximately 5 days.

61 The method of claim 1, wherein said first period of time is approximately 6 days.

62 The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.

63 The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.

64 The method of claim I. wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.

65 The method of claim 1, wherein said total amount of said produced a-ABeta is at least 1 fold higher that the amount of a-ABeta produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

66 The method of claim 1, wherein said total amount of said produced a-ABeta is at least 2-fold higher that the amount of a-ABeta produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
93
"61: ThFni'6thbdodclaim 1, wherein said cell culture is further provided with supplementary components.

68 The method of claim 67, wherein said supplementary components are provided at multiple intervals.
69 The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.

70 A method of producing a-ABeta in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of. i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 rnM; maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.
94
mAo"8'6' f"priloducing a-ABeta in a large-scale production cell culture comprising steps of,
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 niM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutainine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM; maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of

culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

72 A method of producing a-ABeta in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least four medium characteristic selected from the group consisting of- i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a
95
id* 6fab65f2GI/IAO% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that u-ABeta accumulates in the cell
culture,

73 A method of producing a-ABeta in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that a-ABeta accumulates in the cell culture.

74 A method of producing a-ABeta in a large-scale production cell culture comprising the steps of.
providing a cell culture comprising;
mammalian cells that contain a gene encoding a-ABeta, which gene is expressed under condition of cell culture; and
96
a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture
were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture
conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that a-ABeta accumulates in the cell culture.

75 The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of(i) a starting amino acid concentration greater than about 70 mM, (ii) a molar starting glutainine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.

76 The method of any one of claims 1-6 or 70-75, wherein:
lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;
ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and
total amount of produced a-ABeta is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

77 The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said a-ABeta.
97
. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said a-ABeta.

79 The method of claim 1. wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.

80 The method of claim 1. wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.

81 The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.

82 The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.

83 The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.

84 The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
98

'Thd"ffiR

56d'o
f Claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

86 The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.

87 The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

88 The method of claim I, wherein said medium has a medium characteristic selected from the group consisting of:
(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;
(v) a cumulative total amount of phenylalanine per unit volume greater than
approximately 2.5 mM;
(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;
(vii) a cumulative total amount of tryptophan per unit volume greater than
approximately 1.0 mM;
(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix.) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.
99
. The'-ffi e-thod of claim 1, wherein said medium has a medium characteristic selected from the group consisting of(i) an initial amount of histidine per unit volume greater than approximately
1.7 mM;
(ii) an initial amount of isoleucine per unit volume greater than approximately
3.5 mM;
(iii) an initial amount of leucine per unit volume greater than approximately
5.5 mM;
(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;
(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) an initial amount of proline per unit volume reater than approximately 2.5
mm;
(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) an initial amount of tyrosine per unit volume greater than approximately
2.0 mM; and
(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.

90 The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.

91 The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 10 mM.

92 The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

93 The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
100
. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.

95 The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.

96 The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.

97 The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.

98 The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately I mM.

99 The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L. 100. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L. 101. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 7 mg/L. 102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L. 103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mgAL. 104. The method of claim I 'wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.
101
I'65.0""'The"ifiRh6d of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L. 106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L. 107. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L.
108. Themethodofclaimlwhereinthecumulativetotalamountofthiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.
102

2/7/9 (Item 5 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01337859
COMBINATION THERAPY FOR DIABETES, OBESITY, AND CARDIOVASCULAR DISEASES
USING GDF-8 INHIBITORS
TRAITEMENT COMBINE DU DIABETE, DE L'OBESITE ET DE MALADIES CARDIOVASCULAIRES, FAISANT APPEL A DES INHIBITEURS DE GDF-8
Patent Applicant/Assignee:
WYETH, Five Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
TOBIN James F, 78 Clearwater Road, Newton, MA 02462, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
FERGUSON Mary K (agent), Finnegan, Henderson, Farabow, Garrett & Dunner,

L.L.P., 901 New York Avenue, Washington, DC 20001-4413, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 200620884 A2-A3 20060223 (WO 0620884)
Application: WO 2005US28766 20050811 (PCT/WO US2005028766)
Priority Application: US 2004600784 20040812
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KM KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NG NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SM SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LT LU LV MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Main International Patent Class (v7): none

International Patent Class (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
A61K-0038/18 A N F B 20060101 H EP
A61K-0045/06 A N L B 20060101 H EP
A61K-0039/395 A N L B 20060101 H EP
Publication Language: English
Filing Language: English
Fulltext Word Count: 21333

English Abstract
A method of treating obesity, cardiovascular diseases, and disorders of insulin metabolism in a subject, comprising administering to the subject a therapeutically effective amount of a GDF-8 inhibitor, and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

French Abstract
La presente invention se rapporte a une methode permettant de traiter l'obesite, des maladies cardiovasculaires et des troubles du metabolisme de l'insuline chez un sujet. La methode selon l'invention consiste a administrer au sujet une dose therapeutiquement efficace d'un inhibiteur de GDF-8, et une dose therapeutiquement efficace d'au moins un autre agent therapeutique permettant de traiter le syndrome cible.

Legal Status (Type, Date, Text)
Publication 20060223 A2 Without international search report and to be republished upon receipt of that report.
Search Rpt 20060427 Late publication of international search report
Republication 20060427 A3 With international search report.
Republication 20060427 A3 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Claim
A method of treating a targeted syndrome in a subject, comprising administering to the subject a therapeutically effective amount of at least one %GDF%-8 inhibitor, and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

2 A method according to claim 1 , wherein the targeted syndrome is chosen from at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism.

3 A method according to claim 1, wherein the %GDF%-8 inhibitor is chosen from
at least one of an antibody against %GDF%-8, an antibody against %GDF%-8 receptor, a modified soluble receptor, a protein binding to %GDF%-8, a protein binding to %GDF%-8 receptor, inhibitors of protease activation of the %GDF%-8 small latent complex, and %GDF%-8 inhibiting mimetics thereof.

4 A method according to claim 3, wherein the %GDF%-8 inhibitor specifically binds a mature %GDF%-8 protein.
5 The method according to claim 1, wherein the therapeutic agent is chosen
from at least one of an angiotensin converting enzyme (ACE) inhibitor, a sulfonylurea agent, an antilipemic agent, a biguanide agent, a thiazolidinedione agent, insulin, an alpha-glucosidase inhibitor, an aldose reductase inhibitor, or a PTPase inhibitor.

6 The method of claim 5, wherein the angiotensin converting enzyme (ACE) inhibitor is chosen from at least one of quinapril, ramipril, verapamil, captopril, diltiazem, clonidine, hydrochlorthiazide, benazepril, prazosin, fosinopril, lisinopril, atenolol, enalapril, perindropril, perindropril tertbutylamine, trandolapril and moexipril, and the suitable pharmaceutically acceptable salt forms thereof.
54
. The method of claim 5, wherein the sulfonylurea agent is chosen from at least one of glipizide, glyburide (glibenclamide), chlorpropamide, tolbutamide, tolazamide and glimepiride, and the pharmaceutically acceptable salt forms thereof.

8 The method of claim 5, wherein the antilipemic agent is chosen from at least one of bile acid sequestrants, fibric acid derivatives, HMG-CoA reductase inhibitors and nicotinic acid compounds, and the pharmaceutically acceptable salt forms thereof.

9 The method of claim 5, wherein the biguanide agent is chosen from at least one of metformin and its pharmaceutically acceptable salt forms.

10 The method of claim 5, wherein the thiazolidinedione agent is chosen from
at least one of pioglitazone and rosiglitazone, and the pharmaceutically acceptable salt forms thereof.

11 The. method of claim 5, wherein the insulin is chosen from at least one of rapid acting insulins, intermediate acting insulins, long acting insulins and combinations of intermediate and rapid acting insulins.

12 The method of claim 5, wherein the alpha-glucosidase inhibitor is chosen
from at least one of miglitol and acarbose, andthe pharmaceutically acceptable salt forms thereof.

13 The method of claim 5, wherein the aldose reductase inhibitor is chosen
from at least one of:
a) a spiro-isoquinoline-pyrrolidine tetrone compound;
b) 2-[(4-bromo fluorophenyl)methyl] fluoro- (9CI);
c) Tolrestat,
d) Sorbinil;
e) Methosorbinil;
f) Zopolrestat;
g) Epalrestat;
h) Zenarestat;
i) Imirestat;
55
j) Ponalrestat;
k) ONO-2235;
1) GP-1447;
m) CT-1 12;
n) BAL-ARI 8;
o) AD-5467;
p) ZD5522;
q) 3,4-dihydro-2,8-diisopropyl thioxo-2H-1 4-benzoxazine acetic acid;
r) 1-[(3-bromo benzofuranyl)sulfonyl]-2,4-imidazolidinedione (M16209): NZ-314, which is 1-Imidazolidineacetic acid, 3-[(3 nitrophenyl)methyl]-2,4,5-trioxo- (9CI);
s) 1 -phthalazineacetic acid, 3,4- dihydro oxo- 3-[[5-trifluoromethyl)

2-benzothiazolyl]methyl]-;
t) M-79175;
u) SP R-21 0;
v) Spiro[pyrrolidine-3,6'(5'H)-pyrrolo[1,2,3-de][1,4]benzoxazine]-2,5,5'
trione, 8'-chloro-2',3'-dihydro- (9Cl);
w) 6-fluoro-2,3-dihyro-2',5'-dioxo-(2S-cis)-spiro[4H benzopyran-4, 4'
imidazolidine] carboxyamide; and'
x) analogs and pharmaceutically acceptable salts thereof.

14 The method of claim 5, wherein the PTPase inhibitor is chosen from at least one compound with the formula (1):
R,
S
Y1 R3
X. Y
I/ 2
R2-X
3
R, is C(O)OR7, 5- to 6-membered heterocycle, H, halogen, CN, or C(O)NR7R8;
R2 is C(O)ZR4 or CN;
56
Z is -O- or -NR5-;
X is CI-3alkylene-, -NR8-CI-3alkylene-, -S-CI-3alkylene-,
-SO-CI3alkylene-, -SO2-CI-3alkylene-, -CI-4alkylene-, -C2-4alkenylene-, or -C2
4alkynylene-, wherein any of the alkylene, alkenylene and alkynylene groups can be optionally substituted with one or more halogen, oxo, HN=, CN, OCF3, OH, NH2, NO2, R4, or Q;
each Y1, Y2, Y3@ Y4, and Y5 is, independently, CR3, N, S, or 0, one or two of
YI, Y2, Y3, Y4, and Y5 can be absent;
each R3 is, independently, H, aryl, 5- to 8-membered heterocyclyl, Cj 6alkyl, C2-6alkenyl, C2-6alkynyl, halogen, CN, OCF3, OH, NH2, NO2, or Q, wherein any of the aryl, heterocyclic, alkyl, alkenyl or alkynyl groups is
optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2s NO2, N3, R4, or Q;
each Q is, independently, -OC(O)NR4R5, -OR4, -OC(O)R4, -COOR4, -C(O)NR4R5, -C(O)R4, -C(=N-OH)R4, -NR4R5, -N+R4R5R6, -NR4C(O)R5, NR4C(O)NR5R6, -NR4C(O)OR5, -NR4S(0)2R5, -SR4, -S(O)R4, -S(0)2R4, or S(0)2NR4R5;
each R4, R5, and R6 is, independently, H, CI-16alkyl, C2-12alkenyl, C212alkynyl, C3-8cycloalkyl, cycloalkylCI-6alkyl, 5- to 8-membered heterocycle, heterocycliCCI-6alkyl, aryl, arylCI-6alkyl, arylC2-6alkenyl, or arylC2-6alkynyl, each R4, R5, and R6 can be optionally substituted with one or more Cj6alkyl, C2-6alkenyl, C2-6alkynyl, halogen, oxo, CN, OCF3, OH, NH2, NO2, N3, -OC(O)NR7R8, -OR7, -OC(O)R7, -COOR7, -C(O)NR7R8, -C(O)R7, -NR7R8, N+R7R8R9,-NR7C(O)R8, -NR7C(O)NR8R9, -NR7C(O)OR8, -NR7S(0)2R8,
SR7, -S(O)R7, -S(0)2R7, or -S(0)2NR7R8;
each R7, R8, and R9 is, independently, H, CI-12alkyl, C2-12alkenyl, C212alkynyl, C3-12CYC[oalkyl, aryl, or arylCI-12alkyl, each R7, R8, and R9 can be
optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2@ or N02;
57
when the ring system is 1-benzothiophene, RI is C(O)OCH3, and X is OCH2-, then R2 is not C(O)OCH3;
when the ring system is 1-benzothiophene, RI is C(O)OH, and X is -OCH2-, then R2 is not C(O)OH;
when the ring system is thieno[2,3-b]pyridine, RI is isopropyl ester, and X is
-OCH2-, then R2 is not C1-3alkyl ester;
when the ring system is thieno[2,3-b]pyridine, RI is C(O)OCI-4alkyl, and X
is -OCH2- or -OCH(CH3)-, then R2 is not CN;
when the ring system is thieno[2,3-b]pyridine, RI is isopropyl ester, and X is
-SCH2CH2-, then R2 is not CN; and
when the ring system is thieno[2,3-b]pyridine, RI is isopropyl ester, and X is -SCH2-, then R2 is not isopropyl ester.

15 The method of claim 5, wherein the PTPase inhibitor is chosen from at least one compound with the formula (11):
RI S
R4
x '10@
R3
R2-@,@ 0
Y
RI is R5, OR5, C(O)OR5, C(O)R5, or C(O)NR5R6;
R2 is R5;
X is CI-3alkylene-, -NR8-CI-3alkylene-, -S-CI-3alkylene-,
-SO-CI-3alkylene-, -SO2-CI-3alkylene-, -CI-4alkylene-, -C2-4alkenylene-, or
-C2-4alkynylene-, wherein any of the alkylene, alkenylene or alkynylene groups can be optionally substituted with one or more halogen, oxo, imido,
CN, OCF3, OH, NH2, NO2, or Q;
58
Y is absent, , or -NR6-;
R3 is H, halogen, CN, CF3, OCF3, CI-3 alkyl, C34CYCloalkyl, CI-3alkoxy, or
aryl;
R4 is A-B-E-D, where A is absent or arylene, heteroarylene, C1-6alkylene, C2-6 alkenyldiyl, or C2-ralkynyl, each A can be optionally substituted with one or more Of CI-6alkyl, C2-6alkenyl, C2-6alkynyl, halogen, CN, OCF3, OH,
NH2, CHO, NO2, or Q, any of the alkyl, alkenyl or alkynyl groups is optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2Y NO2, N3, or Q;
each A can be optionally terminated with one or more arylene, alkylene, -or
alkenylene;
B is absent or -NR5-, -NR7-, -N(R5)CH2-, -N(R7)CH2-, -N(Rg)-, -N(Rq)C(O)-,
-N(Rq)C(O)C(Rjj)(R12)-, -N(Rg)C(O)C(O)-7 -N(Rq)C(0)N(RIo)-j -N(R9)SO2-, -N(Rq)S02C(RIo)(Rjj)-@ -N(Rq)(Rjo)C(Rjj)(R12)-,
-N(Rq)C(Rjj)(R12)C(R13)(R14)-, , C(RjI)(RI2)-,
-O-C(RIj)(R12)C(Rj3)(RI4)-, -C(RII)(R12)-O-, -C(Rjj)(R12)-O-C(RI3)(R14)-,

C(Rjj)(R12)N(Rq)-, -C(Rjj)(RI2)N(Rq)C(R13)(R14)-, -C(RII)(R12)S-, -C(RII)(R12)SC(R13)(RI4)-, or -C(RIj)(RI2)S02C(R13)(R14)-;
E is absent or C3-12CYCloalkylene, 3-to 12- membered heterocycdiyl, arylene, CI-12alkylene, C2-12alkenylene, or C2-12alkynylene, where each E is optionally substituted with one or more CI-3alkyl, CI-3alkoxy, halogen,
CN, OH, NH2, or N02;
D is one or more H, halogen, OH, NH2, CHO, CN, N02, CF3, or Q;
when A, B, and E are absent, RI is C(O)OH or C(O)OCH3, R2 is H, and R3 is H or chlorine, D is not H or chlorine; and when A, B, and E are absent,
RI is C(O)OH or C(O)OCH3, R2 is H, and R3 is H or bromine, D is not H or bromine;
each Q, independently, is -R5, -R7, -OR5, -OR7, -NR,5R6, -NR5R7, .N+R5R6R8, S(O)nR5, or -S(O)nR7, and n is 0, 1, or 2;
59
each R5, R6, and R8, independently, is H, C1-12alkyl, C2-12alkenyl, C212alkynyl, C3-12CYCloalkyl, CI-12alkoxyCI-12alkyl, cycloalkylCI-6alkyl, 3- to 8membered heterocycyl, heterocycylCI-6alkyl, aryl, arylCI-6alkyl, arylC2-6
alkenyl, or arylC2-6 alkynyl, each R5, R6, and R8 can be optionally substituted with one or more R9, -OR9, -OC(O)OR9, -C(O)Rg, C(O)OR93
-C(O)NRgRIo2 -SR9, -S(O)Rg, -S(O)2R9j -NR9Rio$ -N+RgRIoRIlg
-NR9C(O)Rio, -NC(O)NRgRIo, -NR9S(O)2Rio, oxo, halogen, CN, OCF3, CF3, OH, or N02;
R7 is -C(O)R5, -C(O)OR5, -C(O)NR5R6, -S(0)2R5, -S(O)R5, or -S(0)2NR5R6;
each R9, Rio, Rij, R12, R13 and R14 is, independently, H, CI-12alkyl,

C2-12alkenyl, C2-12alkynyl, C3-12CYCloalkyl, aryl, or arylCI-12alkyl, any of the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or arylalkyl groups is optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2, or NO2.

16 The method of claim 1 , wherein the administration is sequential.

17 The method of claim 1, wherein the administration is simultaneous.

18 The method of claim 1, wherein the administration of at least one therapeutic agent is oral.

19 The method of claim 1, wherein the administration is parenteral.
20 The method of claim 19, wherein the parenteral administration is intravenous.

21 A pharmaceutical composition useful for treating a targeted syndrome comprising combining a therapeutically effective amount of a %GDF%-8 inhibitor,and a therapeutically effective amount of at least one other therapeutic agent w hich treats the targeted syndrome.

22 A pharmaceutical composition according to claim 21, wherein the %GDF%-8
inhibitor is chosen from at least one of an antibody against %GDF%-8, an antibody against %GDF%-8 receptor, a.modified soluble receptor, a protein binding to %GDF%-8, a protein binding to %GDF%-8 receptor, inhibitors of 60
protease activation of the %GDF%-8 small latent complex, and %GDF%-8 inhibiting mimetics thereof.

23 A pharmaceutical composition according to claim 22 wherein the protein binding to %GDF%-8 is chosen from at least one of a %GDF%-8 propeptide having SEQ ID NO:65, a mutated %GDF%-8 propeptide, follistatin, follistatin-domain containing proteins, and Fc fusions thereof.
24 A pharmaceutical composition according to claim 22, wherein the %GDF%-8
inhibitor specifically binds a mature %GDF%-8 protein.

25 The pharmaceutical composition according to claim 21 , wherein the therapeutic agent is chosen from at least one of an angiotensin converting
enzyme (ACE) inhibitor, a sulfonylurea agent, an antilipemic agent, a biguanide agent, a thiazolidinedione agent, insulin, an alpha-glucosidase inhibitor, an aldose reductase inhibitor, or a PTPase inhibitor.

26 The pharmaceutical composition of claim 25, wherein the angiotensin converting enzyme (ACE) inhibitor is chosen from at least one of quinapril, ramipril, verapamil, captopril, diltiazem, clonidine, hydrochlorthiazide, benazepril, prazosin, fosinopril, lisinopril, atenolol, enalapril, perindropril,
perindropril tert-butylamine, trandolapril and moexipril, or a pharmaceutically acceptable salt form of one or more of these compounds.

27 The pharmaceutical composition of claim 25, wherein the sulfonylurea agent is chosen from at least one of glipizide, glyburide (glibenclamide),
chlorpropamide, tolbutamide, tolazamide and glimepriride, and the pharmaceutically acceptable salt forms thereof.

28 The pharmaceutical composition of claim 25, wherein the antilipemic agent is chosen from at least one of bile acid sequestrants, fibric acid derivatives,
HMG-CoA reductase inhibitors and nicotinic acid compounds, and the pharmaceutically acceptable salt forms thereof.
61
. The pharmaceutical composition of claim 25, wherein the biguanide agent is chosen from at least one of metformin and its pharmaceutically acceptable salt forms.

30 The pharmaceutical composition of claim 25, wherein the
thiazolidinedione
agent is chosen from at least one of pioglitazone and rosiglitazone, and pharmaceutically acceptable salt forms of these agents.

31 The pharmaceutical composition of claim 25, wherein the insulin is chosen from at least one of rapid acting insulins, intermediate acting insulins, long acting insulins and combinations of intermediate and rapid acting insulins.
32 The pharmaceutical composition of claim 25, wherein the alphaglucosidase inhibitor is chosen from at least one of miglitol and acarbose,
and a pharmaceutically acceptable salt form of one or more of these compounds.

33 The pharmaceutical composition of claim 25, wherein the aldose reductase
inhibitor is chosen from at least one of
a) a spiro-isoquinoli.ne-pyrrolidine tetrone compound;
b) 2-[(4-bromo fluorophenyl)methyl] fluoro- (9CI);
c) Tolrestat,
d) Sorbinil;
e) Methosorbinil;
f) Zopolrestat;
g) Epalrestat;
h) Zenarestat;
i) Imirestat;
j) Ponalrestat;
k) ONO-2235;
1) GP-1447;
m) CT-1 12;
n) BAL-ARI 8;
o) AD-5467;
p) ZD5522;
62
q) 3,4-dihydro-2,8-diisopropyl thioxo-2H-1,4-benzoxazine acetic acid;
r) 1+3-bromo benzofuranyl)sulfonyl]-2,4-imidazolidinedione (M16209): NZ-314, which is 1-Imidazolidineacetic acid, 3+3 nitrophenyi)methyll-2,4,5-trioxo- (9CI);
s) 1 -phthalazineacetic acid, 3,4- dihydro oxo- 3-[[5-trifluoromethyl) 2-benzothiazolyl]methyl]-;
t) M-79175;
u) SP R-21 0;
v) Spiro[pyrrolidine-3,6'(5'H)-pyrrolo[1 2,3-de][1,4]benzoxazinel-2,5,5' trione, 8'-chloro-2',3'-dihydro- (9CI);
w) 6-fluoro-2,3-dihyro-2',5'-dioxo-(2S-cis)-spiro[4H benzopyran-4, 4' imidazolidine] carboxyamide;
analogs and pharmaceutically acceptable salts thereof.

34 The pharmaceutical composition of claim 25, wherein the PTPase inhibitor
is chosen from at least one compound with the formula (1):
RI S
Y1 R3
xy
R2-X IIs/
Y
3
Y@
RI is C(O)OR7, 5- to 6-membered heterocycle, H, halogen, CN, or C(O)NR7R8;
R2 is C(O)ZR4 or CN;
Z is -O- or -NR5-;
X is CI-3alkylene-, -NR8-CI-3alkylene-, -S-CI-3alkylene-, -SO-Cj3alkylene-, -SO2-CI-3alkylene-, -CI-4alkylene-, -C2-4alkenylene-, or -C2
4alkynylene-, wherein any of the alkylene, alkenylene and alkynylene groups can be optionally substituted with one or more halogen, oxo, HN=, CN, OCF3, OH, NH2, N02, R4, or Q;
63

each Yj 1 Y2, Y33 Y4, and Y5 is, independently, CR3, N, S, or 0, one or two of
yl@ Y2@ Y3i Y4, andy5can be absent;
each R3 is, independently, H, aryl, 5- to 8-membered heterocyclyl, Cj 6alkyl, C2-6alkenyl, C2-6alkynyl, halogen, CN, OCF3, OH, NH2, NO2, or Q, wherein any of the aryl, heterocyclic, alkyl, alkenyl or alkynyl groups is
optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH23 N02, N3, R4, or Q;
each Q is, independently, -OC(O)NR4R5, -OR4, -OC(O)R4, -COOR4, -C(O)NR4R5, -C(O)R4, -C(=N-OH)R4, -NR4R5, -N'R4R5R6, -NR4C(O)R5, NR4C(O)NR5R6, -NR4C(O)OR5, -NR4S(0)2R5, -SR4, -S(O)R4, -S(0)2R4,or S(0)2NR4R5;
each R4, R5, and R6 is, independently, H, Cl-16alkyl, C2-12alkenyl, C212alkynyl, C3-8CYCloalkyl, cycloalkylCl-6alkyl, 5- to 8-membered heterocycle, heterocyclicC,-6alkyl, aryl, arylCl-6alkyl, arylC2-6alkenyl, or arylC2-6alkynyl, each R4, R5, and R6 can be optionally substituted with one or more Cj6alkyl, C2-6alkenyl, C2-6alkynyl, halogen, oxo, CN, OCF3, OH, NH2, NOZ N3,
--OC(O)NR7R8, -OR7, -OC(O)R7, -COOR7, -C(O)NR7R8, -C(O)R7, -NR7R8, N'R7R8R9,-NR7C(O)R8, -NR7C(O)NR8R9, -NR7C(O)OR8, -NR7S(0)2R8, SR7, -S(O)R7, -S(0)2R7,or -S(0)2NR7R8;
each R7, R8, and R9 is, independently, H, Cl-12alkyl, C2-12alkenyl, C212alkynyl, C3-12CYCloalkyl, aryl, or arylCl-12alkyl, each R7, R8, and R9 can be
optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2i or N02;
when the ring system is 1-benzothiophene, Rl is C(O)OCH3, and X is OCH2-, then R2 is not C(O)OCH3;
when the ring system is 1-benzothiophene, Rl is C(O)OH, and X is -OCH2-, then R2 is not C(O)OH;
when the ring system is thieno[2,3-b]pyridine, Rl is isopropyl ester, and X is
-OCH2-, then R2 is not Cl-3alkyl ester;
64
when the ring system is thieno[2,3-b]pyridine, Rl is C(O)OC14alkyl, and X is -OCH2- or -OCH(CH3)-, then R2 is not CN;
when the ring system is thieno[2,3-b]pyridine, Rl is isopropyl ester, and X is
-SCH2CH2-, then R2 is not CN; and
when the ring system is thieno[2,3-b]pyridine, Rl is isopropyl ester, and X is -SCH2-, then R2 is not isopropyl ester.

35 The pharmaceutical composition of claim 25, wherein the PTPase inhibitor
is chosen from at least one compound with the formula (11):
Rl S
R4
."oo@
x
R3
R2 @@ oe@ 0
Y
Rl is R5, OR5, C(O)OR5, C(O)R5, or C(O)NR5R6;
R2 is R5;
X is Cl-3alkylene-, -NR8-Cl-3alkylene-, -S-Cl-3alkylene-, -SO-Cl-3alkylene-, -SO2-Cl-3alkylene-, -Cl-4alkylene-, -C2-4alkenylene-, or
-C2-4alkynylene-, wherein any of the alkylene, alkenylene or alkynylene groups can be optionally substituted with one or more halogen, oxo, imido,
CN, OCF3, OH, NH2, NO2, or Q;
Y is absent, , or -NR6-;
R3 is H, halogen, CN, CF3, OCF3, Cl-3 alkyl, C3-4CYCloalkyl, Cl-3alkoxy, or
aryl;
R4 is A-B-E-D, where A is absent or arylene, heteroarylene, Cl-6alkylene, C2-r,.alkenyldiyl, or C2-6alkynyl, each A can be optionally substituted with one or more Of C1-6alkyl, C2-6alkenyl, C2-6alkynyl, halogen, CN, OCF3, OH@
65
NH2, CHO, NO2, or Q, any of the alkyl, alkenyl or alkynyl groups is optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2@ NO2, N3, or Q;
each A can be optionally terminated with one or more arylene, alkylene, or
alkenylene;
B is absent or -NR5-, -NR7-, -N(R5)CH2-, -N(R7)CH2-, -N(Rg)-, -N(Rg)C(O)-,
-N(Rq)C(O)C(Rjj)(R12)-, -N(Rq)C(O)C(O)-j -N(Rq)C(O)N(Rjo)-, -N(Rg)SO2-i
-N(Rq)SO2C(Rj0)(Rlj)-, -N(Rq)(Rjo)C(Rjj)(R12)-,
-N(Rq)C(Rll)(R12)C(R13)(R14)-, -O.; -O-C(Rjl)(Rl2)-,
-O-C(Rjl)(R12)C(R13)(R14)-, -C(Ril)(Rl2)-O-, -C(Rjj)(R12)-O-C(Rl3)(R14)-,
-C(Rjj)(R12)N(Rq)-, -C(Rjj)(R12)N(Rq)C(R13)(R14)-, -C(Rij)(R12)S-,
-C(Rjj)(Rl2)SC(R13)(R14)-, or-C(Rjj)(R12)SO2C(Rj3)(R14)-;
E is absent or C3-12CYCloalkylene, 3-to 12- membered heterocycdiyl, arylene, Cl-12alkylene, C2-12alkenylene, or C2-12alkynylene, where each E is optionally substituted with one or more Cl-3alkyl, C1-3alkoxy, halogen,
CN, OH, NH2, or N02;
D is one or more H, halogen, OH, NH2, CHO, CN, NO2, CF3, or Q;
when A, B, and E are absent, Rl is C(O)OH or C(O)OCH3, R2 is H, and R3 is H or chlorine, D is not H or chlorine; and when A, B, and E are absent, Rl is C(O)OH or C(O)OCH3, R2' is H, and R3 is H or bromine, D is not H or
bromine;
each Q, independently, is -R5, -R7, -OR5, -OR7, -NR5R6, -NR5R7, -N+R5R6R8, S(O),R5, or -S(O),R7, and n is 0, 1 , or 2;
each R5, R6, and R8, independently, is H, Cl-12alkyl, C2-12alkenyl, C212alkynyl, C3-12CYCloalkyl, Cl-12alkoxyCl-12alkyl, cycloalkylCl-6alkyl, 3- to 8membered heterocycyl, heterocycylCl-6alkyl, aryl, arylCl-6 alkyl, arylC2-6
alkenyl, or arylC2-6 alkynyl, each R5, R6, and R8 can be optionally substituted with one or more R9, -OR9, -OC(O)OR9, -C(O)Rq, -C(O)OR9, -C(O)NR9Rjo1 -SR9, -S(O)R9, -S(O)2Rqj -NRqRjo1 -N+RqRj0Rll,
66
-NRgC(O)Rlo, -NC(0)NRqRio, -NRgS(0)2R,o, oxo, halogen, CN, OCF3, CF3, OH, or N02;
R7 is -C(O)R5, -C(O)OR5, -C(O)NR5R6, -S(0)2R5, -S(O)R5, or -S(0)2NR5R6;
each R9, Rio, Rii, R12, R13 and R14 is, independently, H, C1-12alkyl, C212alkenyl, C2-12alkynyl, C3-12cycloalkyl, aryl, or arylCl-12alkyl, any of the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or arylalkyl groups is optionally substituted with one or more halogen, oxo, CN, OCF3, OH, NH2, or N02.
67

2/7/10 (Item 6 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01239053
REMOVAL OF HIGH MOLECULAR WEIGHT AGGREGATES USING HYDROXYAPATITE
CHROMATOGRAPHY
ELIMINATION D'AGREGATS DE POIDS MOLECULAIRE ELEVE AU MOYEN DE LA CHROMATOGRAPHIE D'ADSORPTION SUR GEL D'HYDROXYAPATITE
Patent Applicant/Assignee:
WYETH, 5 Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
SUN Shujun, 43 Peabody Drive, Brentwood, NH 03833, US, US (Residence), US (Nationality), (Designated only for: US)
GALLO Christopher, 9 Karen Road, Windham, NH 03087, US, US (Residence), US (Nationality), (Designated only for: US)
KELLEY Brian, 108 Blakely Road, Medford, MA 02155, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
GARRETT Arthur S (agent), Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street N.W., Washinton, D.C. 20005-3315, US,

Patent and Priority Information (Country, Number, Date):
Patent: WO 200544856 A2-A3 20050519 (WO 0544856)
Application: WO 2004US32883 20041006 (PCT/WO US04032883)
Priority Application: US 2003514018 20031027; US 2003523335 20031120
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Main International Patent Class (v7): C07K-016/06
International Patent Class (v7): B01D-015/08
Publication Language: English
Filing Language: English
Fulltext Word Count: 14377

English Abstract
This invention relates to the application of hydroxyapatite chromatography to the purification of at least one antibody from a preparation containing high molecular weight aggregates. Further, this invention relates to an integration of ceramic hydroxyapatite chromatography into a combination chromatographic protocol for the removal of high molecular weight aggregates from an antibody preparation.

French Abstract
La presente invention a trait a l'application de la chromatographie sur gel d'hydroxyapatite pour la purification d'au moins un anticorps derive d'une preparation contenant des agregats a poids moleculaire eleve. L'invention a egalement trait a une integration de la chromatographie sur ceramique d'hydroxyapatite dans un protocole de combinaison chromatographique pour l'elimination d'agregats a poids moleculaire eleve d'une preparation d'anticorps.

Legal Status (Type, Date, Text)
Publication 20050519 A2 Without international search report and to be republished upon receipt of that report.
Search Rpt 20050623 Late publication of international search report
Republication 20050623 A3 With international search report.
Republication 20050623 A3 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Claim

1 . A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising:
(a) contacting the antibody preparation with a hydroxyapatite resin and eluting purified antibody from the resin with at least one elution
buffer comprising from 1 to 20 mM sodium phosphate and from 0.2 to 2.5 M NaCl; or
(b) contacting a hydroxyapatite resin with an antibody preparation in load buffer comprising from 1 to 20 mM sodium phosphate and from 0.2 to 2.5 M NaCl and allowing the purified antibody to flow through the column.

2 The method of claim 1 , wherein the purified antibody contains less than 5% high molecular weight aggregates.

3 The method of claim 1, wherein the purified antibody contains less than I % high molecular weight aggregates.

4 The method of claim 1, wherein the elution buffer or load buffer has a pH from 6.4 to 7

5 The method of claim 4, wherein the elution buffer or load buffer contains 3 mM or 5 mM sodium phosphate.

. The method of claim 4, wherein the elution buffer or load buffer contains 1 M or 0.35 M NaCl.

7 The method of claim 4, wherein the elution buffer or load buffer has a pH of 6.8 or 7

8 The method of claim 1, wherein the antibody is an IgG, IgA, IgD, IgE, or IgM antibody.

9 The method of claim 1, wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or a fragment thereof.

10 The method of claim 1 , wherein the antibody is an anti-IL-21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-13, or antiIL-22 antibody.

11 The method of claim 1 , wherein the antibody has a basic pl.

12 The method of claim 1, wherein the resin is ceramic hyd roxyapatite type I or type II.

13 The method of claim 12, wherein the resin is ceramic hydroxyapatite type 1.

. The method of claim 1, wherein the purified antibody contains less than 300 ppm Protein A.

15 A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising subjecting the antibody preparation to (a) Protein A affinity chromatography,
(b) ion exchange chromatography, and (c) hydroxyapatite chromatography,
(i) wherein the purified antibody is eluted from the hydroxyapatite resin using an elution buffer comprising from 1 to 20 mM sodium phosphate and from 0.2 to 2.5 M NaCl; or
(ii) contacting a hydroxyapatite resin with an antibody preparation in load buffer comprising from 1 to 20 mM sodium phosphate and from 0.2 to 2.5 M NaCl and allowing the purified antibody to flow through the column.

16 The method of claim 15, wherein the Protein A affinity chromatography is performed first and the hydroxyapatite chromatography is performed last.

17 The method of claim 15, wherein the purified antibody contains less than 5% high molecular weight aggregates.

18 The method of claim 15, wherein the purified antibody contains less than 1 % high molecular weight aggregates.

. The method of claim 15, wherein the elution buffer or load buffer has a pH from 6.4 to 7

20 The method of claim 19, wherein the elution buffer or load buffer contains 3 mM or 5 mM sodium phosphate.

21 The method of claim 19, wherein the elution buffer or load buffer contains 1 M or 0.35 M NaCl.

22 The method of claim 19, wherein the elution buffer or load buffer has a pH of 6.8 or 7

23 The method of claim 15, wherein the antibody is an IgG, IgA, IgD, IgE, or IgM antibody.

24 The method of claim 15, wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or fragment thereof.

25 The method of claim 15, wherein the antibody is ananti-IL-21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-13, or antiIL-22 antibody.

26 The method of claim 15, wherein the antibody has a basic pl.

. The method of claim 15, wherein the resin is ceramic

hydroxyapatite type I or type IL

28 The method of claim 27, wherein the resin is ceramic hydroxyapatite type 1.

29 The method of claim 15, wherein the ion exchange chromatography is anion exchange chromatography.

30 The method of claim 15, wherein the purified antibody contains less than 300 ppm Protein A.

31 A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising:
(a) contacting the preparation with a Protein A support;
(b) allowing the antibody to adsorb to the Protein A support;
(c) washing the Protein A support and adsorbed antibody with at least one Protein A washing buffer;
(d) eluting the adsorbed antibody with at least one Protein A elution buffer;
(e) contacting the Protein A eluate with an ion exchange support;
(f) allowing the antibody to flow through the ion exchange support;
(g) washing the ion exchange support with at least one ion exchange washing buffer;
(h) contacting the ion exchange flow-through with a hydroxyapatite resin;
(i) allowing the antibody to adsorb to the resin;
0) washing the resin with at least one hydroxyapatite washing buffer; and
(k) eluting purified antibody from the resin with at least one hydroxyapatite elution buffer comprising from 1 to 20 mM sodium phosphate and from 0.2 to 2.5 M NaCl.

32 The method of claim 31, wherein the purified antibody contains less than 5% high molecular Weight aggregates.

33 The method of claim 31, wherein the purified antibody contains less than 1 % high molecular weight aggregates.

34 The method of claim 31, wherein the at least one hydroxyapatite elution buffer has a pH from 6.4 to 7

35 The method of claim 34, wherein the at least one hydroxyapatite elution buffer contains 3 mM or 5 mM sodium phosphate.

36 The method of claim 34, wherein the at least one hydroxyapatite elution buffer contains 0.35 M or 1.0 M NaCl.
, The method of claim 34, wherein the at least one hydroxyapatite elution buffer has a pH of 6.8 or 7

38 The method of claim 31, wherein the antibody is an IgG, IgA, IgD, IgE, or IgM antibody.

39 The method of claim 31 , wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or a fragment thereof.

40 The method of claim 31, wherein the antibody is an anti-IL-21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-1 3, or antiIL-22 antibody.
41 The method of claim 31, wherein the antibody has a basic pl.

42 The method of claim 31, wherein the resin is ceramic hydroxyapatite type I or type II.

43 The method of claim 42, wherein the resin is ceramic hydroxyapatite type 1.

44 The method of claim 31, wherein the ion exchange chromatography is anion exchange chromatography.

. The method of claim 31, further comprising filtering the ion exchange flow-through before application to the hydroxyapatite resin, thereby reducing the viral contaminants.

46 The method of claim 31, further comprising subjecting the hydroxyapatite eluate to at least one of ultrafiltration or diafiltration.

47 The method of claim 31 , wherein the purified antibody contains less than 300 ppm Protein A.
1
48 A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising:
(a) contacting the preparation with a Protein A support;
(b) allowing the antibody to adsorb to the Protein A support;
(c) washing the Protein A support and adsorbed antibody with at least one Protein A washing buffer;
(d) eluting the adsorbed antibody with at least one Protein A elution buffer;
(e) contacting the Protein A eluate with an ion exchange support;
(f) allowing the antibody to flow through the ion exchange support;
(g) washing the ion exchange support with at least one ion exchange washing buffer;
(h) exchanging the ion exchange flow-through into a load buffer comprising from I to 20 mM sodium phosphate and from 0.2 to 2.5 M NaCl;
(i) contacting the ion exchange flow-through with a hydroxyapatite resin;
0) allowing the antibody to flow through the hydroxyapatite resin; and
(k) washing the hydroxyapatite resin with at least one hydroxyapatite washing buffer,
49 The method of claim 48, wherein the purified antibody contains less than 5% high molecular weight aggregates.

50 The method of claim 48, wherein the purified antibody contains less than 1 % high molecular weight aggregates.

51 The method of claim 48, wherein the load buffer has a pH from 6.4 to 7

52 The method of claim 51, wherein the load buffer contains 5 mM sodium phosphate.

53 The method of claim 51, wherein the load buffer contains 350 mM NaCl.

54 The method of claim 51, wherein the load buffer has a pH of 6
. The method of claim 48, wherein the antibody is an IgG, IgA, IgD, IgE, or IgM antibody.

56 The method of claim 48, wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or a fragment thereof.
57 The method of claim 48, wherein the antibody is an anti-IL-21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-1 3, or antiIL-22 antibody.

58 The method of claim 48, wherein the antibody has a basic pl.

59 The method of claim 48, wherein the resin is ceramic hyd roxyapatite type I or type II.

60 The method of claim 59, wherein the resin is ceramic hydroxyapatite type 1.

61 The method of claim 48, wherein the ion exchange chromatography is anion exchange chromatography.

62 The method of claim 48, further comprising filtering the ion

exchange flow-through before application to the hydroxyapatite resin, thereby reducing the viral contaminants.
, The method of claim 48, further comprising subjecting the hydroxyapatite flow-through to at least one of ultrafiltration or diafiltration.
64 The method of claim 48, wherein the purified antibody contains less than 300 ppm Protein A.

65 A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising:
(a) equilibrating hydroxyapatite resin with at least one equilibration buffer comprising from 1 to 20 mM sodium phosphate and from 0.01 to 2.0 M NaCl;
(b) contacting the antibody preparation with said hydroxyapatite resin under conditions that allow binding of both monomer and high molecular
weight aggregates;
(c) allowing the high molecular weight aggregates to bind more tightly than antibody monomers and, as loading continues, allowing the high
molecular weight aggregates to displace bound monomer; and
(d) collecting said displaced monomer.

66 The method of claim 65, wherein the purified antibody contains less than 5% high molecular weight aggregates.

67 The method of claim 65, wherein the purified antibody contains less than 1 % high molecular weight aggregates.
. The method of claim 65, wherein the equilibration buffer has a pH from 6.2 to 8

69 The method of claim 68, wherein the equilibration buffer contains 2 mM or 5 mM sodium phosphate.

70 The method of claim 68, wherein the equilibration buffer contains 50 mM or 100 mM NaCl.

71 The method of claim 68, wherein the equilibration buffer has a pH of 7 1

72 The method of claim 68, wherein the equilibration buffer further comprises up to 200 mM arginine or HEPES.
t

73 The method of claim 68, wherein the equilibration buffer further comprises 1 00 mM or 120 mM arginine and 1 00 mM or 20 mM HEPES.

74 The method of claim 65, wherein the antibody is an IgG, IgA7 IgD, IgE, or IgM antibody.
75 The method of claim 65, wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or a fragment thereof.
. The method of claim 65, wherein the antibody is an anti-IL-21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-1 3, or antiIL-22 antibody.

77 The method of claim 65, wherein the antibody has a basic pl.

78 The method of claim 65, wherein the resin is ceramic hydroxyapatite type I or type II.

79 The method of claim 78, wherein the resin is ceramic hydroxyapatite type 1.

80 The method of claim 65, wherein the purified antibody contains less than 300 ppm Protein A.

81 A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising subjecting the antibody preparation to (a) Protein A affinity chromatography,
(b) ion exchange chromatography, and (c) hydroxyapatite chromatography, wherein (i) the hydroxyapatite resin is equilibrated with at least one equilibration buffer comprising from 1 to 20 mM sodium phosphate
and from 0.01 to 2.0 M NaCl;
(ii) the antibody preparation is contacted with said hydroxyapatite resin under conditions that allow binding of both monomer and
high molecular weight aggregates;
(iii) the high molecular weight aggregates are allowed to bind more tightly than antibody monomers, and as loading continues, the high molecular weight aggregates are allowed to displace bound monomer; and
(iv) said displaced monomers are collected.

82 The method of claim 81, wherein the Protein A affinity chromatography is performed first and the hydroxyapatite chromatography is performed last.

83 The method of claim 81, wherein the purified antibody contains less than 5% high molecular weight aggregates.

84 The method of claim 81, wherein the purified antibody contains less than 1 % high molecular weight aggregates.
85 The method of claim 81, wherein the equilibration buffer has a pH from 6.2 to 8

86 The method of claim 85, wherein the equilibration buffer contains 2 mM or 5 mM sodium phosphate.

87 The method of claim 85, wherein the equilibration buffer contains 1 00 mM or 50 M NaCl.
. The method of claim 85, wherein the elution buffer or load buffer has a pH of 7

89 The method of claim 85, wherein the equilibration buffer further comprises up to 200 mM arginine or HEPES.

90 The method of claim 85, wherein the equilibration buffer further comprises 1 00 mM or 120 mM arginine and 1 00 mM or 20 mM HEPES.

91 The method of claim 81, wherein the antibody is an IgG, IgA, IgD, IgE, or IgM antibody.

92 The method of claim 81 , wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or fragment thereof.
93 The method of claim 81, wherein the antibody is an anti-IL-21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-13, or antiIL-22 antibody.

94 The method of claim 81, wherein the antibody has a basic pl.

95 The method of claim 81, wherein the resin is ceramic hydroxyapatite type I or type 11.
. The method of claim 95, wherein the resin is ceramic hydroxyapatite type 1.

97 The method of claim 81, wherein the ion exchange chromatography is anion exchange chromatography.

98 The method of claim 81, wherein the purified antibody contains less than 300 ppm Protein A.

99 A method for purifying at least one antibody from an antibody preparation containing high molecular weight aggregates comprising:
(a) contacting the preparation with a Protein A support;
(b) allowing the antibody to adsorb to the Protein A support;
(c) washing the Protein A support and adsorbed antibody with at least one Protein A washing buffer;
(d) eluting the adsorbed antibody with at least one Protein A elution buffer;

(e) contacting the Protein A eluate with an ion exchange support;
(f) allowing the antibody to flow through the ion exchange support;
(g) washing the ion exchange support with at least one ion exchange washing buffer;
(h) equilibrating hydroxyapatite resin with at least one equilibration buffer comprising from 1 to 20 mM sodium phosphate and from .01 to 2.0 M NaCl;
(i) contacting the ion exchange flow-through with said hydroxyapatite resin under conditions that allow binding of both monomer and
high molecular weight aggregates;
allowing the high molecular weight aggregates to bind more tightly than antibody monomers, and as loading continues, allowing the high
molecular weight aggregates to displace bound monomer;
(k) washing the hydroxyapatite resin with at least one hydroxyapatite washing buffer; and
(1) collecting said displaced monomer.
100. The method of claim 99, wherein the purified antibody contains less than 5% high molecular weight aggregates.
101. The method of claim 99, wherein the purified antibody contains less than 1 % high molecular weight aggregates.
102. The method of claim 99, wherein the equilibration buffer has a pH from 6.2 to 8
103. The method of claim 102, wherein the equilibration buffer contains 2'mM or 5 mM sodium phosphate.
104. The method of claim 102, wherein the equilibration buffer contains 1 00 mM or 50 M NaCl.
. The method of claim 102, wherein the equilibration buffer has a pH of 7
106. The method of claim 102, wherein the equilibration buffer further comprises up to 200 mM arginine or HEPES.
107. The method of claim 102, wherein the equilibration buffer further comprises 1 00 mM or 120 mM arginine and 1 00 mM or 20 mM HEPES.
108. The method of claim 99, wherein the antibody is an IgG, IgA, IgD, IgE, or IgM antibody.
109. The method of claim 99, wherein the antibody is monoclonal, polyclonal, chimeric, humanized, or fragment thereof. 110. The method of claim 99, wherein the antibody is an anti-IL21 receptor, anti-%GDF%-8, anti-Abeta, anti-CD22, anti-Lewis Y, anti-IL-13, or anti-IL-22 antibody.
Ill. The method of claim 99, wherein the antibody has a basic pl.
112. The method of claim 99, wherein the resin is ceramic hydroxyapatite type I or type II.
. The method of claim I 1 2, wherein the resin is ceramic hydroxyapatite type 1.
114. The method of claim 99, wherein the ion exchange chromatography is anion exchange chromatography.
115. Themethodofc[aim99,furthercomprisingfilteringtheion exchange flow-through before application to the hydroxyapatite resin, thereby reducing the viral contaminants.
116. Themethodofclaim99,furthercomprisingsubjectingthe hydroxyapatite eluate to at least one of ultrafiltration or diafiltration,
117. The method of claim 99, wherein the purified antibody contains less than 300 ppm Protein A.

2/7/11 (Item 7 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01188197
USE OF MYOSTATIN (GDF8) INHIBITORS IN CONJUNCTION WITH CORTICOSTEROIDS FOR
TREATING NEUROMUSCULAR DISORDERS
METHODES DE TRAITEMENT ET DE PREVENTION DES TROUBLES NEUROMUSCULAIRES

Patent Applicant/Assignee:
WYETH, Five Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
WHITTEMORE Lise-Anne, 11 Tanglewood Road, East Walpole, Norfolk, MA 02032 , US, US (Residence), US (Nationality),
LI Xiangping, 30 Rice Road, Wayland, MA 01778, US, US (Residence), US (Nationality),
Legal Representative:
GARRETT Arthur S (agent), Finnegan, Henderson, Farrabow Garrett & Dunner, L.L.P., 901 New York Avenue, NW, Washington, D.C. 20001-4413, US
Patent and Priority Information (Country, Number, Date):
Patent: WO 2004108157 A2-A3 20041216 (WO 04108157)
Application: WO 2004US17049 20040601 (PCT/WO US2004017049)
Priority Application: US 2003474603 20030602
Designated States:
(All protection types applied unless otherwise stated - for applications 2004+)
AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) BW GH GM KE LS MW MZ NA SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Main International Patent Class (v7): A61K-039/395
International Patent Class (v7): A61P-021/00

International Patent Class (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
A61K-0039/395 A I F B 20060101 H EP
A61P-0021/00 A I L B 20060101 H EP
Publication Language: English
Filing Language: English
Fulltext Word Count: 7102

English Abstract
The disclosure provides methods for treating neuromuscular disorders in mammals. The disclosed methods include administering therapeutically effective amounts of a %GDF%-8 inhibitor and a corticosteroid to a subject susceptible to, or having, a neuromuscular disorder, so as to maintain desirable levels of muscle function.

French Abstract
L'invention concerne des methodes de traitement des troubles neuromusculaires chez des mammiferes. Les methodes selon l'invention consistent a administrer des quantites therapeutiquement efficaces d'un inhibiteur de %GDF%-8 et d'un corticosteroide a un sujet souffrant d'un trouble neuromusculaire ou susceptible de developper un tel trouble, de sorte a maintenir la fonction musculaire a un niveau desire.

Claim
1 A method of treating a mammal with a decrease of muscle function, comprising administering to the mammal a therapeutically effective amount of at least one %GDF%-8 inhibitor and a therapeutically effective amount of at least one corticostbroid in the amounts and for a period of time sufficient to treat decrease of muscle function.

2 The method of claim 1, wherein the muscle function of at least one muscle is evaluated by at least one parameter chosen from muscle mass, muscle contraction force, serum CK concentration, or muscle morphology.

3 The method of claim 1 , wherein the muscle whose function is treated is chosen from at least one of gastrocnemius, tibialis anterior, quadriceps, extensor digitorum longus, cardiac muscle, or diaphragm muscle.

4 The method of claim 1, wherein treating said mammal results in

increased body weight of said mammal.

5 The method of claim 1 , wherein treating said mammal results in increased grip strength.

6 A method of treating muscle weakness, comprising administering to a mammal a therapeutically effective amount of at least one GIDF-8 inhibitor and a therapeutically effective amount of at least one corticosterold in the amounts and for a period of time sufficient to treat loss of musple strength.

7 A method of treating corticosteroid-induced muscle atrophy, comprising administering to a mammal a therapeutically effective amount of at least one %GDF%-8 inhibitor sufficient to treat the corticosteroid-induced muscle atrophy.
28
. A method of treating a neuromuscular disorder, comprising administering to a mammal having or at risk of the neuromuscular disorder a
therapeutically effective amount of at least one %GDF%-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat the neuromuscular disorder.

9 The method of claim 8, wherein the neuromuscular disorder is a muscular dystrophy.
1 0. The method of claim 9, wherein the muscular dystrophy is Duchenne muscular dystrophy.
1 1. The method of claim 9, wherein the muscular dystrophy is Becker muscular dystrophy.

12 The method as in any one of claims I -1 I , wherein the mammal is human.

13 The method as in any one of claims I -1 1, wherein the corticosteroid is chosen from at least one of:
(a) at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide;
(b) a derivative of at least one of beclomethasone diproplonate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide; or
(c) a pharmaceutically acceptable salt of at least one of beclomethasone dipropionate, budesonide, cortisol,
29
dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetoniden.

14 The method as in any one of claims 1-1 1, wherein the corticosterold is prednisone or prednisolone.

15 The method as in any one of claims 1 -1 1, wherein the corticosteroid is administered at a dose between 0.1 and 2.0 mg/kg/day.

16 The method as in any one of claims I -1 1, wherein the corticosteroid is administered orally.
17 The method as in any one of claims 1 -1 1 , wherein the %GDF%-8 inhibitor is chosen from an antibody to %GDF%-8, an antibody to a %GDF%-8 receptor, a soluble %GDF%-8 receptor, a %GDF%-8 propeptide, a small molecule
inhibitor of %GDF%-8, follistatin, or a follistatin-domain-containing protein.

18 The method of claim 17, wherein the antibody to %GDF%-8 is chosen from JA-1 6, %Myo29%, Myo28, or Myo22.

19 The method of claim 17, wherein the %GDF%-8 propeptide is mutated at an aspartate residue.

20 The method of claim 17, wherein the %GDF%-8 propeptide is joined to the Fc portion of an immunoglobulin.

21 The method of claim 17, wherein the %GDF%-8 receptor is ActRIIB.

22 The method of claim 17, wherein the %GDF%-8 receptor is joined to the Fc portion of an immunoglobulin.

23 The method of claim 17, wherein the %GDF%-8 inhibitor is follistatin.
30
. The method of claim 17, wherein the follistatin-domain-containing protein is GASP

25 The method of claim 17, wherein the %GDF%-8 inhibitor is a small molecule inhibitor.

25 The method of claim 1, wherein the method results in treating of cardiomyopathy of said mammal.

26 The method of claim 1, wherein the administration of %GDF%-8 inhibitor and corticosteroid is concurrent.

27 The method of claim 1, wherein the administration of %GDF%-8 inhibitor and corticosteroid is consecutive.

28 The method of claim 8, wherein the administration of %GDF%-8 inhibitor and corticosteroid is concurrent.

29 The method of claim 8, wherein the administration of %GDF%-8 inhibitor and corticosteroid is consecutive.
31

2/7/12 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

01116993
NEUTRALIZING ANTIBODIES AGAINST %GDF%-8 AND USES THEREFOR
ANTICORPS DE NEUTRALISATION DIRIGES CONTRE %GDF% -8 ET UTILISATIONS DE CEUX-CI
Patent Applicant/Assignee:
WYETH, Five Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
CAMBRIDGE ANTIBODY TECHNOLOGY, The Milstein Building, Granta Park, Cambridge CB1 6GH, GB, GB (Residence), GB (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
VELDMAN Geertruida M, 60 Woodmere Drive, Sudbury, MA 01776, US, US (Residence), US (Nationality), (Designated only for: US)
DAVIES Monique V, 167 Indian Rest Road, Harpswell, MA 04079, US, US (Residence), US (Nationality), (Designated only for: US)
SONG Kening, 10 Patrick Street, Arlington, MA 02474, US, US (Residence), US (Nationality), (Designated only for: US)
WOLFMAN Neil M, 5 Phillips Lane, Dover, MA 02030, US, US (Residence), US (Nationality), (Designated only for: US)
GROVE-BRIDGES Kristie, 9 George Road, Maynard, MA 01754, US, US (Residence), US (Nationality), (Designated only for: US)
FIELD Anne, 7 Arningford Crescent, Melbourn, Royston, Hertsfordshire, SG8 6NG, GB, GB (Residence), GB (Nationality), (Designated only for: US)
RUSSELL Caroline, 40 Redwing Rise, Royston, Hertsfordshire SG8 7XU, GB, GB (Residence), GB (Nationality), (Designated only for: US)
VALGE-ARCHER Viia, 36 West Field, Little Abington, Cambridgeshire CB1 6BE , GB, GB (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
GARRETT Arthur S (agent), Finnegan, Henderson, Farrabow Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC 20005-3315, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200437861 A2-A3 20040506 (WO 0437861)
Application: WO 2003IB4748 20031022 (PCT/WO IB03004748)
Priority Application: US 2002419964 20021022

Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Main International Patent Class (v7): C07K-016/22
International Patent Class (v7): A61K-039/395; A61P-003/00; A61P-021/00
Publication Language: English
Filing Language: English
Fulltext Word Count: 17569

English Abstract
The disclosure provides novel antibodies against growth and differentiation factor-8 (%GDF%-8), in particular human antibodies, and antibody fragments, including those that inhibit %GDF%-8 activity in vitro and/or in vivo. The disclosure also provides methods for diagnosing, preventing, or treating degenerative disorders of muscle or bone, or disorders of insulin metabolism.

French Abstract
L'invention concerne de nouveaux anticorps diriges contre le facteur de croissance et de differenciation 8 (%GDF%-8), en particulier des anticorps humains, et des fragments d'anticorps, y compris ceux qui inhibent l'activite de %GDF%-8 in vitro et/ou in vivo. L'invention concerne aussi des methodes permettant de diagnostiquer, de prevenir ou de traiter des troubles degeneratifs des muscles ou des os, ou des troubles du metabolisme de l'insuline.

Legal Status (Type, Date, Text)
Publication 20040506 A2 Without international search report and to be republished upon receipt of that report.
Search Rpt 20040722 Late publication of international search report
Republication 20040722 A3 With international search report.
Republication 20040722 A3 Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Claim
1 An isolated antibody comprising an amino acid sequence substantially as set out in SEQ ID NO:n, wherein n is 2, 41 61 8, 102 129 14, 162 18, 20, 223 24, 26, 28, 30, 317 32t 33, 34, 35, 367

37 38, 399 40, 41, 429 43@ 44@ 459 46, 47, or 48; and wherein the antibody is capable of specifically binding %GDF%-8 or BMP-1 1.

2 The antibody of claim 1 , comprising the amino acid sequence of SEQ ID NO:n, wherein n is 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24@ 26, 28@ 30, 31, 32, 33, 34, 35@ 36, 37@ 38, 392 409 419 42, 43,

44 45, 46, 47, or 48.

3 The antibody of claim 1 , wherein said antibody is an scFv fragment expressed by E. coli having ATCC Deposit Designation No. PTA-4741, PTA-4740, or PTA

4 The antibody of claim 1, wherein the antibody is capable of specifically binding to a protein comprising the amino acid sequence set forth in SEQ ID NO:54.

5 The antibody of claim 4, wherein at least (a) the second amino acid from the N-terminus of SEQ ID NO:54 is methionine, (b) the third amino acid from the N-terminus is serine, or (c) the fifth amino acid from the N-terminus is isoleucine, independently of each other.

6 The antibody of claim 1, wherein the antibody is human.

7 The antibody of claim 1 , wherein the antibody is IgG, or IgG4
62
. The antibody of claim 1 , wherein the amino acid sequence of the antibody is modified to reduce or alter effector function.

9 The antibody of claim 8, wherein the amino acid sequence is modified at residues corresponding to amino acid 1 1 7 or amino acid 120 of SEQ ID NO:53.

10 The antibody of claim 1, wherein the antibody is IgGjx or IgG,,,.

11 A pharmaceutical composition, comprising the antibody of claim 1.

12 A method of treatment, comprising administering an effective dose of the pharmaceutical composition of claim 1 1.

13 The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from muscle disorder, neuromuscular disorder, and bone degenerative disorder.

14 The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome, and amyotrophic lateral sclerosis.

15 The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of Duchenne's muscular dystrophy.
63
. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from obesity and adipose tissue disorder.

17 The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, and type 2 diabetes.
18 The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need -of treatment or prevention of type 2 diabetes.

19 The meth od of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of obesity.

20 The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need for repair of damaged muscle.

21 The method of claim 21, wherein the damaged muscle is myocardial muscle.

22 The method of claim 21 , wherein the damaged muscle is diaphragm.

23 The method of claim 12, wherein the antibody is administered at an effective dose chosen from 1 pg/kg to150 mg/kg, 1 pg/kg to
64
00 mg/kg, 1 pg/kg to 50 mg/kg, 1 pg/kg to 20 mg/kg, 1 pg/kg to

10 mg/kg, 1 pg/kg to 1 mg/kg, 10 pg/kg to 1 mg/kg, 10 pg/kg to 1 00 pg/kg, 1 00 pg to 1 mg/kg, and 500 pg/kg to 1 mg/kg.

24 An isolated nucleic acid encoding the antibody of claim 1.

25 An expression vector, comprising the nucleic acid of claim 24.

26 A host cell, comprising the vector of claim 25.

27 The host cell of claim 26, wherein said host cell is E. coli having ATCC Deposit Designation No. PTA4741, PTA-4740, or PTA

28 The nucleic acid of claim 24, wherein the nucleic acid comprises a nucleotide sequence of SEQ ID NO:n, wherein n is 1, 39 51 77 9111, 13,15,179 19, 21, 23, 25, 27, or 29.

29 A method of making an antibody that specifically reacts with %GDF%-8, the method comprises:
(a) providing a starting repertoire of nucleic acids encoding a variable domain which either include a CDR3 to be replaced or lack a CDR3 encoding region;
(b) combining the repertoire With a donor nucleic acid encoding an amino acid sequence substantially as set out in SEQ ID NO:n , where n is an integer from 31 to 48, such that the donor nucleic acid is inserted into the CDR3 region in the repertoire so as to provide a product repertoire of nucleic acids encoding a variable domain;
(c) expressing the nucleic acids of the product repertoire;
65
(d) selecting a specific antigen-binding fragment specific for %GDF%-8; and
(e) recovering the specific antigen-binding fragment or nucleic acid encoding the binding fragment.

30 An antibody produced by the method of claim 29

31 A method for identifying inhibitors of %GDF%-8, comprising:
(a) preparing a first binding mixture comprising the antibody of claim 1 and %GDF%-8;
(b) measuring the amount of binding between the antibody and,%GDF%-8 in the first mixture;
(c) preparing a second binding mixture comprising the antibody, %GDF%-8, a test compound; and
(d) measuring the amount of binding between the antibody and %GDF%-8 in the second mixture.

32 A method of increasing muscle strength or mass, the method comprising administering a therapeutically effective amount of the antibody of claim 1 to a mammal, thereby increasing muscle strength or mass.

33 An isolated antibody against %GDF%-8, wherein the antibody is capable of inhibiting binding of %GDF%-8 to ActRIIB.

34 The antibody of claim 33 comprising the amino acid sequence substantially as set out in SEQ ID NO:n, wherein n is 2, 49 61 8t 10,12,14216718,20,22,24,26,28,30,31,32,33@34,35,36l 377 38, 397 40, 419 42, 43, 44, 45, 46, 47, or 48.
66
. The antibody of claim 33 comprising the amino acid sequence as set out in SEQ ID NO:n, wherein n is 2, 4, 6, 8,10, 12, 142 162 18, 209 22, 24v 26t 28v 30, 31, 32t 33, 34, 352 36, 37, 38v 39,

40 41 9 42, 43@ 449 45, 46, 47, or 48.
36 A method of increasing muscle strength, the method comprising administering a therapeutically effective amount of the antibody of claim 33to a mammal, thereby increasing muscle strength.

37 The antibody of claim 33 wherein the antibody is capable of specifically binding BMP-1 1.

38 A method of making an antibody, comprising culturing E. coli having ATCC Deposit Designation No. PTA-4741, PTA-4740, or PTA-4739 and recovering the antibody.

39 The method of claim 38, further comprising fusing the nucleic acid encoding the svFv of %Myo29%, Myo28, or Myo22 with nucleic acids encoding the Fc portion of an immunoglobulin and expressing the fused nucleic acid in a cell.

40 The method of claim 39, comprising germlining.

41 An antibody made using the method of claim 40.

42 An antibody capable of specifically binding to an epitope characterized by the amino acid sequence set forth in SEQ ID NO:54.

43 Use of the antibody of any of claims 1-10, 31, 34, 35@ 369 38, 41, and 42 for the preparation of a medicament for treatment or
67
prevention of at least one disorder of muscle, bone, or glucose homeostasis in a mammal.

44 The use of claim 43, wherein the mammal is human.

45 The use of claim 43, wherein the disorder is a neuromuscular disorder.

46 The use of claim 43, wherein the disorder is muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome, congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome, -or amyotrophic lateral sclerosis.

47 The use of claim 43, wherein the disorder is obesity or an adipose tissue disorder.
48 The use of claim 43, wherein the disorder is syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, or type 2 diabetes.

49 Use of the antibody of any of claims 1-10, 31, 34, 35, 36, 38, 41, and 42 for the preparation of a medicament for at least one of:
(a) repair of muscle damage, (b) increasing muscle mass or strength, and (c) increasing glucose tolerance in a mammal.

50 The use of claim 49, wherein the damaged muscle of (a) is myocardiac muscle or diaphragm.

51 The use of any one of claims 46-51, wherein the antibody is adminitered to the mammal at an effective dose chosen from 1 Pg/kg to 150 mg/kg, 1 pg/kg to 1 00 mg/kg, 1 pg/kg to 50 mg/kg,
68
pg/kg to 20 mg/kg, 1 pg/kg to 1 0 mg/kg , 1 pg/kg to 1 mg/kg , 1 0 pg/kg to 1 mg/kg, 10 pg/kg to 100 pg/kgl 100 pg to 1 mg/kg, and 500 pg/kg to 1 mg/kg.
69

2/7/13 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0358310 DBR Accession No.: 2005-04014 PATENT
Treating neuromuscular disorder such as muscular dystrophy, decrease of muscle function, or muscle weakness, by administering concurrently or consecutively growth differentiation factor-8 and corticosteroid to mammal - involving vector-mediated gene transfer and expression in mammal cell for therapy

AUTHOR: WHITTEMORE L; LI X
PATENT ASSIGNEE: WYETH 2004
PATENT NUMBER: WO 2004108157 PATENT DATE: 20041216 WPI ACCESSION NO.: 2005-048484 (200505)
PRIORITY APPLIC. NO.: US 474603 APPLIC. DATE: 20030602
NATIONAL APPLIC. NO.: WO 2004US17049 APPLIC. DATE: 20040601
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - Treating (M1) a neuromuscular disorder, decrease of muscle function, or muscle weakness, involves administering at least one growth differentiation factor (%GDF%)-8 and corticosteroid to the mammal, for a time period sufficient to treat the conditions. DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for treating (M2) corticosteroid-induced muscle atrophy, involves administering at least one %GDF%-8 inhibitor to the mammal. BIOTECHNOLOGY - Preferred Method: In (M1), the muscle function of at least one muscle is evaluated by at least one parameter chosen from muscle mass, muscle contraction force, serum creatine kinase concentration, or muscle morphology. The muscle whose function is treated, is chosen from gastrocnemius, tibialis anterior, quadriceps, extensor digitorum longus, cardiac muscle, or diaphragm muscle. The corticosteroid is chosen from (a) at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide, (b) a derivative of any one of the corticosteroid of (a), and (c) a salt of any one of the corticosteroid of (a). The corticosteroid is preferably prednisone or prednisolone. The %GDF%-8 inhibitor is chosen from an antibody to %GDF%-8, and antibody to %GDF%-8 receptor, a soluble %GDF%-8 receptor, %GDF%-8 propeptide, small molecule inhibitor of %GDF%-8, follistatin, and follistatin-domain-containing protein. The antibody to %GDF%-8 is chosen from JA-16, %Myo29%, Myo28, and Myo22. The %GDF%-8 propeptide is mutated at an aspartate residue, or joined to the Fc portion of an immunoglobulin. The %GDF%-8 receptor is ActRIIB, and is joined to Fc portion of an immunoglobulin is preferably follistatin or small molecule inhibitor. The follistatin-domain containing protein is GASP-1. ACTIVITY - Cardiovascular-Gen.; Muscular-Gen. Male C57BL/10ScSn-mdxlj and C57BL/10 mice were used to determine the effect of %GDF%-8 neutralizing antibody and prednisone on dystrophic muscle. Mouse monoclonal anti-%GDF% -8 antibody JA-16, prednisone or vehicle (peanut oil) was injected to 5-7 week old mice for 4 weeks. Mice were intraperitoneally injected with JA-16 at a dose of 60 mg/kg/week, or subcutaneously injected with prednisone at 2 mg/kg, 3 times a week. The body weight and grip strength were monitored before, during and after treatment. Mice were sacrificed and muscle mass was assessed by dissecting and weighing the gastrocnemius and quadriceps. The results show that administration of %GDF%-8 inhibitor together with prednisone effectively decreased muscular dystrophy, and increased muscle mass and strength than in the treatment with prednisone alone or vehicle. MECHANISM OF ACTION - Inhibitor of %GDF%-8 (claimed). No supporting data is given. USE - (M1) is useful for treating a neuromuscular disorder, decrease of muscle function, cardiomyopathy or muscle weakness in a mammal, preferably human. The treatment results in increased body weight and grip strength of the mammal. The neuromuscular disorder is a muscular dystrophy such as Duchenne muscular dystrophy or Becker muscular dystrophy. (M2) is useful for treating corticosteroid-induced muscle atrophy (all claimed). ADMINISTRATION - Corticosteroid is administered orally at a dosage of 0.1-2 mg/kg/day. Corticosteroid and %GDF%-8 inhibitor are administered concurrently or consecutively (claimed). The %GDF%-8 inhibitor is administered at a dosage of 1 microgram/kg to 25 mg/kg. (33 pages)

2/7/14 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0342038 DBR Accession No.: 2004-14330 PATENT
New antibody that binds %GDF%-8 or BMP-11, useful in preparing a composition for repairing damaged muscle, increasing muscle mass or strength or increasing glucose tolerance in a mammal - antibody production via plasmid expression in host cell for use in disease therapy
AUTHOR: VELDMAN G M; DAVIES M V; SONG K; WOLFMAN N M; GROVE-BRIDGES K; FIELD A; RUSSELL C; VALGE-ARCHER V
PATENT ASSIGNEE: WYETH; CAMBRIDGE ANTIBODY TECHNOLOGY 2004
PATENT NUMBER: WO 200437861 PATENT DATE: 20040506 WPI ACCESSION NO.: 2004-365497 (200434)
PRIORITY APPLIC. NO.: US 419964 APPLIC. DATE: 20021022
NATIONAL APPLIC. NO.: WO 2003IB4748 APPLIC. DATE: 20031022
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A new isolated antibody comprises a sequence having 5-258 amino acids and is capable of specifically binding %GDF% -8 or BMP-11. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a pharmaceutical composition comprising the antibody; (2) a method of treating a disorder; (3) an isolated nucleic acid encoding the antibody; (4) an expression vector comprising the nucleic acid; (5) a host cell comprising the vector; (6) a method of making an antibody that specifically reacts with %GDF%-8; (7) a method for identifying inhibitors of %GDF%-8; (8) a method of increasing muscle strength or mass; and (9) an isolated antibody. BIOTECHNOLOGY - Preferred Antibody: The antibody is an scFv fragment expressed by Escherichia coli having ATCC Deposit Designation No. PTA-4741, PTA-4740 or PTA-4739. It binds to a protein comprising 6-amino acid sequence, where the second amino acid from the N-terminus is methionine, the third amino acid from the N-terminus is serine and the fifth amino acid from the N-terminus is isoleucine. It is human. It comprises IgG1 or IgG4. It is capable of inhibiting binding of %GDF%-8 to ActRIIB. The amino acid sequence of the antibody is modified to reduce or alter effector function. The amino acid sequence is modified at residues corresponding to amino acid 117 or 120 of the 330-amino acid sequence. Preferred Host Cell: The host cell is Escherichia coli having ATCC Deposit Designation No. PTA-4741, PTA-4740 or PTA-4739. Preferred Nucleic Acid: The nucleic acid comprises a sequence having 315-786 bp. Preferred Method: Treating a disorder comprises administering the pharmaceutical composition. The pharmaceutical composition is administered for treating or preventing muscle, neuromuscular or bone degenerative disorder. The disorder comprises muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome, congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome, amyotrophic lateral sclerosis, obesity, adipose tissue disorder, syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, type 2 diabetes or damaged myocardial or diaphragm muscle. Making an antibody that specifically reacts with %GDF% -8 comprises: (1) providing a starting repertoire of nucleic acids encoding a variable domain that either includes a CDR3 to be replaced or lacks a CDR3 encoding region; (2) combining the repertoire with a donor nucleic acid encoding the amino acid sequence; (3) expressing the nucleic acids of the product repertoire; (4) selecting a specific antigen-binding fragment specific for %GDF%-8; and (5) recovering the specific antigen-binding fragment or nucleic acid encoding the binding fragment. Identifying inhibitors of %GDF%-8 comprises: (1) preparing a first binding mixture comprising the antibody and %GDF%-8; (2) measuring the amount of binding between the antibody and %GDF%-8 in the first mixture; (3) preparing a second binding mixture comprising the antibody, %GDF%-8, a test compound; and (4) measuring the amount of binding between the antibody and %GDF%-8 in the second mixture. Increasing muscle strength or mass comprises administering the antibody to a mammal. The method further comprises fusing the nucleic acid encoding the svFv of %Myo29%, Myo28 or Myo22 with nucleic acids encoding the Fc portion of an immunoglobulin and expressing the fused nucleic acid in a cell. The method also comprises germlining. ACTIVITY - Neuroprotective; Muscular-Gen; Respiratory-Gen; Antidiabetic; Osteopathic; Anorectic. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The antibody is useful in preparing a composition for repairing damaged muscle, increasing muscle mass or strength or increasing glucose tolerance in a mammal or for treating or preventing neuromuscular disorder, bone degenerative disorder, muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome, congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome,

amyotrophic lateral sclerosis, obesity, adipose tissue disorder, syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, type 2 diabetes or damaged myocardial or diaphragm muscle (claimed). ADMINISTRATION - Dosage comprises 1mug to 150mg, preferably 500mug to 1mg per kg body weight (claimed). The composition is administered via oral or parenteral route. EXAMPLE - No relevant examples given. (117 pages)

2/7/15 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

145417005 CA: 145(21)417005q PATENT
Detection of an immune response to GDF-8 modulating agents
INVENTOR(AUTHOR): Nowak, John A.; O'Hara, Denise M.; Cryan, John G.; Caiazzo, Teresa M.; Joyce, Alison; Rajewski, Joseph W., III; Sun, Shujun; Wolfman, Neil M.
LOCATION: USA
ASSIGNEE: Wyeth, John, and Brother Ltd.
PATENT: PCT International ; WO 2006107611 A2 DATE: 20061012
APPLICATION: WO 2006US10711 (20060323) *US 2005PV664643 (20050323)
PAGES: 75pp. CODEN: PIXXD2 LANGUAGE: English
PATENT CLASSIFICATIONS:
CLASS: G01N-000/A
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BW; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; EG; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KM; KN; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; LY; MA; MD; MG; MK; MN; MW; MX; MZ; NA; NG; NI; NO; NZ; OM; PG; PH; PL; PT; RO; RU; SC; SD; SE; SG; SK; SL; SM; SY; TJ; TM; TN; TR; TT; TZ; UA; UG; US; UZ; VC; VN; YU; ZA DESIGNATED REGIONAL: AT; BE; BG; CH ; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR; HU; IE; IS; IT; LT; LU; LV; MC; NL; PL; PT; RO; SE; SI; SK; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG; BW; GH; GM; KE; LS; MW; MZ; NA; SD; SL; SZ; TZ; UG; ZM; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM
SECTION:
CA215001 Immunochemistry
IDENTIFIERS: immunoassay growth differentiation factor inhibitor antibody , myostatin inhibitor MYO029 antibody immunoassay
DESCRIPTORS:
Antibodies and Immunoglobulins...
conjugates; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Dyes... Enzymes,uses... Epitopes... Fluorescent substances... Luminescent substances... Radionuclides...
detection agent; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Amniotic fluid... Animal tissue... Antibodies and Immunoglobulins... Aves ... Avidins... Blood plasma... Blood serum... Blood... Cerebrospinal fluid ... Colostrum... Fish... Gastric juice... Human... Immunoassay... Lymph... Mammalia... Milk... Mucus... Reptilia... Saliva... Sweat... Synovial fluid ... Tear(ocular fluid)... Urine...
detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Immunoassay...
enzyme-linked immunosorbent assay; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Proteins...
GASP-1, modulating agent; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Receptors...
GDF-8, modulating agent; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Antibodies and Immunoglobulins...
immobilized; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Nucleic acids...
modulating agent; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
Activin receptors...
type IIB, modulating agent; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
CAS REGISTRY NUMBERS:
58-85-5 54827-17-7 271597-12-7 705287-60-1 detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
9003-99-0 horseradish; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
117628-82-7 modulating agent; detection of antibodies to growth differentiation factor-8 modulating agents such as MYO-029
911528-79-5 911528-81-9 911528-83-1 911528-85-3 911528-87-5 unclaimed nucleotide sequence; detection of an immune response to GDF-8 modulating agents
911528-78-4 911528-80-8 911528-82-0 911528-84-2 911528-86-4 911528-88-6 911763-45-6 unclaimed protein sequence; detection of an immune response to GDF-8 modulating agents
558426-67-8 558426-68-9 685832-67-1 685832-69-3 685832-70-6 685832-72-8 unclaimed sequence; detection of an immune response to GDF-8 modulating agents

2/7/16 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

142033042 CA: 142(3)33042u PATENT
Therapeutic and prophylactic methods using a GDF-8 inhibitor and a corticosteroid for neuromuscular disorders
INVENTOR(AUTHOR): Whittemore, Lise-Anne; Li, Xiangping
LOCATION: USA
ASSIGNEE: Wyeth, John, and Brother Ltd.
PATENT: PCT International ; WO 2004108157 A2 DATE: 20041216
APPLICATION: WO 2004US17049 (20040601) *US 2003PV474603 (20030602)
PAGES: 33 pp. CODEN: PIXXD2 LANGUAGE: English
PATENT CLASSIFICATIONS:
CLASS: A61K-039/395A
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BW; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; EG; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NA; NI; NO; NZ; OM; PG; PH; PL; PT; RO; RU; SC; SD; SE; SG; SK; SL; SY; TJ; TM; TN; TR; TT; TZ; UA; UG; US; UZ; VC; VN; YU; ZA; ZM; ZW DESIGNATED REGIONAL: BW; GH; GM; KE; LS; MW; MZ ; NA; SD; SL; SZ; TZ; UG; ZM; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM; AT; BE; BG; CH; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR; HU; IE; IT; LU; MC; NL; PL; PT; RO; SE; SI; SK; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG
SECTION:
CA201012 Pharmacology
IDENTIFIERS: muscle disorder treatment GDF 8 inhibitor corticosteroid
DESCRIPTORS:
Muscle,disease...
atrophy, corticosteroid-induced; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscular dystrophy...
Becker's; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle...
cardiac; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Heart,disease...
cardiomyopathy; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscular dystrophy...
Duchenne; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle...
extensor digitorum longus; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Proteins...
follistatin domain-contg.; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Antibodies and Immunoglobulins...

fusion products; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Proteins...
GASP-1; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle...
gastrocnemius; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle,disease... Corticosteroids,biological studies... Combination chemotherapy... Abdominal diaphragm... Neuromuscular diseases... Muscular dystrophy... Human... Cardiovascular agents... Drug interactions...
Prophylaxis...
GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Receptors...
GDF-8; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Antibodies and Immunoglobulins...
monoclonal, JA-16, Myo29, Myo28, and Myo22; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Mutagenesis...
mutated GDF-8 propeptide; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Heart...
myocardium; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Antibodies and Immunoglobulins...
neutralizing, to GDF-8; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Drug delivery systems...
oral; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle...
quadriceps; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle...
tibial, anterior; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Antibodies and Immunoglobulins...
to GDF-8; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Activin receptors...
type IIB; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
Muscle,disease...
weakness; GDF-8 inhibitor and corticosteroid for neuromuscular disorders
CAS REGISTRY NUMBERS:
5534-09-8D 51333-22-3D 50-23-7D 50-02-2D 80474-14-2D 83919-23-7D 53-03-2D 76-25-5D derivs., GDF-8 inhibitor and corticosteroid for neuromuscular disorders
271597-12-7 5534-09-8 51333-22-3 50-23-7 50-02-2 80474-14-2 83919-23-7 53-03-2 76-25-5 50-24-8 117628-82-7 GDF-8 inhibitor and corticosteroid for neuromuscular disorders
271597-12-7D propeptides, GDF-8 inhibitor and corticosteroid for neuromuscular disorders

2/7/17 (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.

6653859
Derwent Accession: 2006-204413
UTILITY
Production of alpha-ABeta
Inventor: Drapeau, Denis, Salem, NH, US
Luan, Yen-Tung, Chelmsford, MA, US
Mercer, James R., Derry, NH, US
Wang, Wenge, North Chelmsford, MA, US
Lasko, Daniel R., Medford, MA, US
Assignee: Wyeth Research Ireland Limited, (03), Newbridge, IE
Correspondence Address: CHOATE, HALL & STEWART LLP, PATENT GROUP, TWO INTERNATIONAL PLACE, BOSTON, MA, 02110, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20060160180	A1	20060720	US 2005213317	20050825
Provisional				US 60-604936	20040827

US Classification on document (Main): 435069100
(X-ref): 435325000; 435455000

International Classification (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
C12P-0021/06 A I F B 20060101 20060720 H US
C12N-0005/06 A I L B 20060101 20060720 H US

Fulltext Word Count: 30066
Number of Claims: 108
Exemplary or Independent Claim Number(s): 1,2,3,4,5,6,24,70,71,72,73,74,76

References to Related Applications:
RELATED APPLICATION
[0001] This application claims priority to Provisional Patent Application No. 60/604,936, filed Aug. 27, 2004, which is incorporated herein by reference in its entirety.

Abstract:
[00000] An improved system for large scale production of proteins and/or polypeptides in cell culture, particularly in media characterized by one or more of: i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of it maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time.

Summary of the Invention:
BACKGROUND OF THE INVENTION
[0002] Proteins and polypeptides have become increasingly important as therapeutic agents. In most cases, therapeutic proteins and polypeptides are produced in cell culture, from cells that have been engineered and/or selected to produce unusually high levels of the particular protein or polypeptide of interest. Control and optimization of cell culture conditions is critically important for successful commercial production of proteins and polypeptides.
[0003] Many proteins and polypeptides produced in cell culture are made in a batch or fed-batch process, in which cells are cultured for a period of time, and then the culture is terminated and the produced protein or polypeptide is isolated. The ultimate amount and quality of protein or polypeptide produced can be dramatically affected by the conditions of the cell culture. For example, traditional batch and fed-batch culture processes often result in production of metabolic waste products that have detrimental effects on cell growth, viability, and production or stability of the protein or polypeptide of interest. While efforts have been made to improve production of proteins and polypeptides in batch and fed-batch culture processes, there remains a need for additional improvements.

[0004] Additionally, significant effort has been invested in the development of defined media (i.e., media assembled from known individual components and lacking serum or other animal byproducts) for use in culturing cells, particularly mammalian cells. Cell growth

characteristics can be very different in defined media as contrasted with serum-derived media. There is a particular need for the development of improved systems for producing proteins and polypeptides by cell culture in defined media.

SUMMARY OF THE INVENTION

[0005] The present invention provides an improved system for large scale production of proteins and/or polypeptides in cell culture. For example, the present invention provides commercial scale (e.g., 500 L or more) culture methods that utilize a medium characterized by one or more of: i) a cumulative amino acid amount per unit volume greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative amount of glutamine and asparagine concentration per unit volume greater than about 16 mM. One of ordinary skill in the art will understand that "cumulative", as used above, refers to the total amount of a particular component or components added over the course of the cell culture, including components added at the beginning of the culture and subsequently added components. In certain preferred embodiments of the invention, it is desirable to minimize "feeds" of the culture over time, so that it is desirable to maximize amounts present initially. Of course, medium components are metabolized during culture so that cultures with the same cumulative amounts of given components will have different absolute levels if those components are added at different times (e.g., all present initially vs. some added by feeds).

[0006] According to the present invention, use of such a medium allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate.

[0007] One of ordinary skill in the art will understand that the media formulations of the present invention encompass both defined and non-defined media. In certain preferred embodiments of the present invention, the culture medium is a defined medium in which the composition of the medium is known and controlled.

[0008] In certain preferred embodiments of the present invention, the culture methods include changing the culture from a first set of culture conditions to a second set of culture conditions so that a metabolic shift of the cells is achieved. In some embodiments, this change is performed when the culture has reached about 20-80% of its maximal cell density. In some embodiments, the change involves changing the temperature (or temperature range) at which the culture is maintained. Alternatively or additionally, the present invention provides methods adjusted so that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time. In other embodiments, the shift involves shifting the pH, osmolarlity or level of chemical inductants, such as alkanoic acids or their salts.

[0009] Cell cultures of the present invention may optionally be supplemented with nutrients and/or other medium components including hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source. In certain embodiments of the present invention, it may be beneficial to supplement the media with chemical inductants such as hexamethylene-bis(acetamide) ("HMBA") and sodium butyrate ("NaB"). These optional supplements may be added at the beginning of the culture or may be added at a later point in order to replenish depleted nutrients or for another reason. In general, it is desirable to select the initial medium composition to minimize supplementation in accordance with the present invention.

[0010] Various culture conditions may be monitored in accordance with the present invention, including pH, cell density, cell viability, lactate levels, ammonium levels, osmolarity, or titer of the expressed polypeptide or protein.

Exemplary or Independent Claim(s):

1. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a medium characteristic selected from the group consisting of: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

2. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

3. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow

said cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

4. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

5. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

6. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

24. The method of claims 18-23, wherein said measurements are taken daily.

70. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

71. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

72. A method of producing [small alpha, Greek]-ABeta in a large-scale

production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least four medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

73. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that [small alpha, Greek]-ABeta accumulates in the cell culture.

74. A method of producing [small alpha, Greek]-ABeta in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding [small alpha, Greek]-ABeta, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20% -80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that [small alpha, Greek]-ABeta accumulates in the cell culture.

76. The method of any one of claims 1-6 or 70-75, wherein:
lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;
ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and
total amount of produced [small alpha, Greek]-ABeta is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

Non-exemplary or Dependent Claim(s):

7. The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.
8. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 13 mM.
9. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 10 mM.
10. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 mM.
11. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.
12. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.
13. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.
14. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.
15. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.
16. The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.
17. The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 [small mu, Greek]M.
18. The method of claim 1, wherein viable cell density of said culture is measured on a periodic basis.
19. The method of claim 1, wherein viability of said culture is measured on a periodic basis.
20. The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.
21. The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.
22. The method of claim 1, wherein said titer of said culture is measured on a periodic basis.
23. The method of claim 1, wherein osmolarity of said culture is measured on a periodic basis.
25. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]2 cells/mL.
26. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]3 cells/mL.
27. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]4 cells/mL.
28. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]5 cells/mL.
29. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]6 cells/mL.
30. The method of claim 1, wherein the initial density of said mammalian cells is at least 5X10[sup]6 cells/mL.
31. The method of claim 1, wherein the initial density of said mammalian cells is at least 10X10[sup]6 cells/mL.
32. The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.
33. The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.
34. The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.
35. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.
36. The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.
37. The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.
38. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees

Celsius.

39. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.
40. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.
41. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.
42. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.
43. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.
44. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.
45. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.
46. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 33 degrees Celsius.
47. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.
48. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.
49. The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.
50. The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.
51. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40 degrees Celsius.
52. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.
53. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.
54. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.
55. The method of claim 1, wherein said first period of time is between 0-8 days.
56. The method of claim 1, wherein said first period of time is between 1-7 days.
57. The method of claim 1, wherein said first period of time is between 2-6 days.
58. The method of claim 1, wherein said first period of time is between 3-5 days.
59. The method of claim 1, wherein said first period of time is approximately 4 days.
60. The method of claim 1, wherein said first period of time is approximately 5 days.
61. The method of claim 1, wherein said first period of time is approximately 6 days.
62. The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.
63. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.
64. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.
65. The method of claim 1, wherein said total amount of said produced [small alpha, Greek]-ABeta is at least 1.5-fold higher that the amount of [small alpha, Greek]-ABeta produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
66. The method of claim 1, wherein said total amount of said produced [small alpha, Greek]-ABeta is at least 2-fold higher that the amount of [small alpha, Greek]-ABeta produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
67. The method of claim 1, wherein said cell culture is further provided with supplementary components.
68. The method of claim 67, wherein said supplementary components are provided at multiple intervals.
69. The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.
75. The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of:
(i) a starting amino acid concentration greater than about 70 mM, (ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.
77. The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said [small alpha, Greek]-ABeta.
78. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said [small alpha, Greek]-ABeta.
79. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.
80. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.
81. The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.
82. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.
83. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.
84. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
85. The method of claim 1, wherein the cumulative total amount of

histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.

86. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
87. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.
88. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:
(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;
(v) a cumulative total amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;
(vii) a cumulative total amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.
89. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:
(i) an initial amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) an initial amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) an initial amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;
(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) an initial amount of proline per unit volume greater than approximately 2.5 mM;
(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) an initial amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.
90. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.
91. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 10 mM.
92. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.
93. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
94. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.
95. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
96. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.
97. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.
98. The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately 1 mM.
99. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L.
100. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L.
101. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 7 mg/L.
102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L.
103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mg/L.
104. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.
105. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L.
106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L.
107. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L.
108. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.

2/7/18 (Item 2 from file: 654)
DIALOG(R)File 654:US Pat.Full.

6590888
Derwent Accession: 2006-240732
UTILITY
Production of TNFR-Ig
Inventor: Drapeau, Denis, Salem, NH, US
Luan, Yen-Tung, Chelmsford, MA, US
Mercer, James R., Derry, NH, US
Wang, Wenge, North Chelmsford, MA, US
Lasko, Daniel R., Medford, MA, US
Assignee: Wyeth Research Ireland Limited, (02), Newbridge, IE
Correspondence Address: CHOATE, HALL & STEWART LLP, PATENT GROUP, TWO INTERNATIONAL PLACE, BOSTON, MA, 02110, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20060121569	A1	20060608	US 2005213633	20050825
Provisional				US 60-605379	20040827

US Classification on document (Main): 435069100
(X-ref): 435334000; 435320100

International Classification (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
C12P-0021/06 A I F B 20060101 20060608 H US
C12N-0005/06 A I L B 20060101 20060608 H US

Fulltext Word Count: 29999
Number of Claims: 108
Exemplary or Independent Claim Number(s): 1,2,3,4,5,6,24,70,71,72,73,74,76

References to Related Applications:

RELATED APPLICATION

[0001] This application claims priority to Provisional Patent Application No. 60/605,379, filed Aug. 27, 2004, which is incorporated herein by reference in its entirety.

Abstract:

[00000] An improved system for large scale production of proteins and/or polypeptides in cell culture, particularly in media characterized by one or more of: i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of it maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time.

Summary of the Invention:

BACKGROUND OF THE INVENTION

[0002] Proteins and polypeptides have become increasingly important as therapeutic agents. In most cases, therapeutic proteins and polypeptides are produced in cell culture, from cells that have been engineered and/or selected to produce unusually high levels of the particular protein or polypeptide of interest. Control and optimization of cell culture conditions is critically important for successful commercial production of proteins and polypeptides.

[0003] Many proteins and polypeptides produced in cell culture are made in a batch or fed-batch process, in -which cells are cultured for a period of time, and then the culture is terminated and the produced protein or polypeptide is isolated. The ultimate amount and quality of protein or polypeptide produced can be dramatically affected by the conditions of the cell culture. For example, traditional batch and fed-batch culture processes often result in production of metabolic waste products that have detrimental effects on cell growth, viability, and production or stability of the protein or polypeptide of interest. While efforts have been made to improve production of proteins and polypeptides in batch and fed-batch culture processes, there remains a need for additional improvements.

[0004] Additionally, significant effort has been invested in the development of defined media (i.e., media assembled from known individual components and lacking serum or other animal byproducts) for use in culturing cells, particularly mammalian cells. Cell growth characteristics can be very different in defined media as contrasted with serum-derived media. There is a particular need for the development of improved systems for producing proteins and polypeptides by cell culture in defined media.

SUMMARY OF THE INVENTION

[0005] The present invention provides an improved system for large scale production of proteins and/or polypeptides in cell culture. For example, the present invention provides commercial scale (e.g., 500 L or more) culture methods that utilize a medium characterized by one or more of: i) a cumulative amino acid amount per unit volume greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative amount of glutamine and asparagine concentration per unit volume greater than about 16 mM. One of ordinary skill in the art will understand that "cumulative", as used above, refers to the total amount of a particular component or components added over the course of the cell culture, including components added at the beginning of the culture and subsequently added components. In certain preferred embodiments of the invention, it is desirable to minimize "feeds" of the culture over time, so that it is desirable to maximize amounts present initially. Of course, medium components are metabolized during culture so that cultures with the same cumulative amounts of given components will have different absolute levels if those components are added at different times (e.g., all present initially vs. some added by feeds).

[0006] According to the present invention, use of such a medium allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate.

[0007] One of ordinary skill in the art will understand that the media formulations of the present invention encompass both defined and non-defined media. In certain preferred embodiments of the present invention, the culture medium is a defined medium in which the composition of the medium is known and controlled.

[0008] In certain preferred embodiments of the present invention, the culture methods include changing the culture from a first set of culture conditions to a second set of culture conditions so that a metabolic shift of the cells is achieved. In some embodiments, this change is performed when the culture has reached about 20-80% of its maximal cell density. In. some embodiments, the change involves changing the temperature (or temperature range) at which the culture is maintained. Alternatively or additionally, the present invention provides methods adjusted so that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time. In other embodiments, the shift involves shifting the pH, osmolarlity or level of chemical inductants, such as alkanoic acids or their salts.

[0009] Cell cultures of the present invention may optionally be supplemented with nutrients and/or other medium components including hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source. In certain embodiments of the present invention, it may be beneficial to supplement the media with chemical inductants such as hexamethylene-bis(acetamide) ("HMBA") and sodium butyrate ("NaB"). These optional supplements may be added at the beginning of the culture or may be added at a later point in order to replenish depleted nutrients or for another reason. In general, it is desirable to select the initial medium composition to minimize supplementation in accordance with the present invention.

[0010] Various culture conditions may be monitored in accordance with the present invention, including pH, cell density, cell viability, lactate levels, ammonium levels, osmolarity, or titer of the expressed polypeptide or protein.

Exemplary or Independent Claim(s):

1. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a medium characteristic selected from the group consisting of: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;

changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

2. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

3. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

4. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

5. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

6. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

24. The method of claims 18-23, wherein said measurements are taken daily.

70. A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;

mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

71. A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

72. A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least four medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

73. A method of producing TNFR-Ig in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that TNFR-Ig accumulates in the cell culture.

74. A method of producing TNFR-Ig in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding TNFR-Ig, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that TNFR-Ig accumulates in the cell culture.

76. The method of any one of claims 1-6 or 70-75, wherein:
lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;
ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and
total amount of produced TNFR-Ig is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

Non-exemplary or Dependent Claim(s):

7. The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.
8. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 13 mM.
9. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 10 mM.
10. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 mM.
11. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.
12. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.
13. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.
14. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.
15. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.
16. The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.

17. The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 [small mu, Greek]M.
18. The method of claim 1, wherein viable cell density of said culture is measured on a periodic basis.
19. The method of claim 1, wherein viability of said culture is measured on a periodic basis.
20. The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.
21. The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.
22. The method of claim 1, wherein said titer of said culture is measured on a periodic basis.
23. The method of claim 1, wherein osmolarity of said culture is measured on a periodic basis.
25. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]2 cells/mL.
26. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]3 cells/mL.
27. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]4 cells/mL.
28. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]5 cells/mL.
29. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]6 cells/mL.
30. The method of claim 1, wherein the initial density of said mammalian cells is at least 5X10[sup]6 cells/mL.
31. The method of claim 1, wherein the initial density of said mammalian cells is at least 10X10[sup]6 cells/mL.
32. The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.
33. The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.
34. The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.
35. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.
36. The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.
37. The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.
38. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.
39. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.
40. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.
41. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.
42. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.
43. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.
44. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.
45. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.
46. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 33 degrees Celsius.
47. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.
48. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.
49. The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.
50. The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.
51. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40 degrees Celsius.
52. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.
53. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.
54. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.
55. The method of claim 1, wherein said first period of time is between 0-8 days.
56. The method of claim 1, wherein said first period of time is between 1-7 days.
57. The method of claim 1, wherein said first period of time is between 2-6 days.
58. The method of claim 1, wherein said first period of time is between 3-5 days.
59. The method of claim 1, wherein said first period of time is approximately 4 days.
60. The method of claim 1, wherein said first period of time is approximately 5 days.
61. The method of claim 1, wherein said first period of time is approximately 6 days.
62. The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.
63. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.
64. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.
65. The method of claim 1, wherein said total amount of said produced TNFR-Ig is at least 1.5-fold higher that the amount of TNFR-Ig produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
66. The method of claim 1, wherein said total amount of said produced TNFR-Ig is at least 2-fold higher that the amount of TNFR-Ig produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
67. The method of claim 1, wherein said cell culture is further provided with supplementary components.
68. The method of claim 67, wherein said supplementary components are provided at multiple intervals.
69. The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.
75. The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of:
(i) a starting amino acid concentration greater than about 70 mM,

(ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.

77. The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said TNFR-Ig.
78. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said TNFR-Ig.
79. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.
80. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.
81. The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.
82. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.
83. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.
84. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
85. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.
86. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
87. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.
88. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:
(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;
(v) a cumulative total amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;
(vii) a cumulative total amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.
89. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:
(i) an initial amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) an initial amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) an initial amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;
(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) an initial amount of proline per unit volume greater than approximately 2.5 mM;
(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) an initial amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.
90. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.
91. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 10 mM.
92. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.
93. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
94. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.
95. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
96. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.
97. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.
98. The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately 1 mM.
99. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L.
100. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L.
101. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 7 mg/L.
102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L.
103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mg/L.
104. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.
105. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L.
106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L.
107. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L.
108. The method of claim 1, wherein the cumulative total amount of

thiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.

2/7/19 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

6590887
Derwent Accession: 2006-240731
UTILITY
Production of polypeptides
Inventor: Drapeau, Denis, Salem, NH, US
Luan, Yen-Tung, Chelmsford, MA, US
Mercer, James R., Derry, NH, US
Wang, Wenge, North Chelmsford, MA, US
Lasko, Daniel R., Medford, MA, US
Assignee: Wyeth Research Ireland Limited, (02), Newbridge, IE
Correspondence Address: CHOATE, HALL & STEWART LLP, PATENT GROUP, TWO INTERNATIONAL PLACE, BOSTON, MA, 02110, US

	Publication Number	Kind	Publication Date	Application Number	Filing Date
Main Patent	US 20060121568	A1	20060608	US 2005213308	20050825
Provisional				US 60-605097	20040827
Provisional				US 60-604941	20040827
Provisional				US 60-605074	20040827

US Classification on document (Main): 435069100
(X-ref): 435325000; 530350000

International Classification (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
C12P-0021/06 A I F B 20060101 20060608 H US
C07K-0014/705 A I L B 20060101 20060608 H US
C12N-0005/06 A I L B 20060101 20060608 H US

Fulltext Word Count: 30049
Number of Claims: 111
Exemplary or Independent Claim Number(s):
1,2,3,4,5,6,24,70,71,72,73,74,76,111

References to Related Applications:
RELATED APPLICATIONS
[0001] This application claims priority to Provisional Patent Application Nos. 60/605,097, 60/604,941, and 60/605,074, each of which was filed Aug. 27, 2004, and each of which is incorporated herein by reference in its entirety

Abstract:
[00000] An improved system for large scale production of proteins and/or polypeptides in cell culture, particularly in media characterized by one or more of: i) a cumulative amino acid concentration greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative glutamine and cumulative asparagine concentration between about 16 and 36 mM, is provided. The use of such a system allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate. Additionally, culture methods including a temperature shift, typically including a decrease in temperature when the culture has reached about 20-80% of it maximal cell density, are provided. Alternatively or additionally, the present invention provides methods such that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time.

Summary of the Invention:
BACKGROUND OF THE INVENTION

[0002] Proteins and polypeptides have become increasingly important as therapeutic agents. In most cases, therapeutic proteins and polypeptides are produced in cell culture, from cells that have been engineered and/or selected to produce unusually high levels of the particular protein or polypeptide of interest. Control and optimization of cell culture conditions is critically important for successful commercial production of proteins and polypeptides.

[0003] Many proteins and polypeptides produced in cell culture are made in a batch or fed-batch process, in which cells are cultured for a period of time, and then the culture is terminated and the produced protein or polypeptide is isolated. The ultimate amount and quality of protein or polypeptide produced can be dramatically affected by the conditions of the cell culture. For example, traditional batch and fed-batch culture processes often result in production of metabolic waste products that have detrimental effects on cell growth, viability, and production or stability of the protein or polypeptide of interest. While efforts have been made to improve production of proteins and polypeptides in batch and fed-batch culture processes, there remains a need for additional improvements.

[0004] Additionally, significant effort has been invested in the development of defined media (i.e., media assembled from known individual components and lacking serum or other animal byproducts) for use in culturing cells, particularly mammalian cells. Cell growth characteristics can be very different in defined media as contrasted with serum-derived media. There is a particular need for the development of improved systems for producing proteins and polypeptides by cell culture in defined media.

SUMMARY OF THE INVENTION

[0005] The present invention provides an improved system for large scale production of proteins and/or polypeptides in cell culture. For example, the present invention provides commercial scale (e.g., 500 L or more) culture methods that utilize a medium characterized by one or more of: i) a cumulative amino acid amount per unit volume greater than about 70 mM; ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; or v) a combined cumulative amount of glutamine and asparagine concentration per unit volume greater than about 16 mM. One of ordinary skill in the art will understand that "cumulative", as used above, refers to the total amount of a particular component or components added over the course of the cell culture, including components added at the beginning of the culture and subsequently added components. In certain preferred embodiments of the invention, it is desirable to minimize "feeds" of the culture over time, so that it is desirable to maximize amounts present initially. Of course, medium components are metabolized during culture so that cultures with the same cumulative amounts of given components will have different absolute levels if those components are added at different times (e.g., all present initially vs. some added by feeds).

[0006] According to the present invention, use of such a medium allows high levels of protein production and lessens accumulation of certain undesirable factors such as ammonium and/or lactate.

[0007] One of ordinary skill in the art will understand that the media formulations of the present invention encompass both defined and non-defined media. In certain preferred embodiments of the present invention, the culture medium is a defined medium in which the composition of the medium is known and controlled.

[0008] In certain preferred embodiments of the present invention, the culture methods include changing the culture from a first set of culture conditions to a second set of culture conditions so that a metabolic shift of the cells is achieved. In some embodiments, this change is performed when the culture has reached about 20-80% of its maximal cell density. In some embodiments, the change involves changing the temperature (or temperature range) at which the culture is maintained.

Alternatively or additionally, the present invention provides methods adjusted so that, after reaching a peak, lactate and/or ammonium levels in the culture decrease over time. In other embodiments, the shift involves shifting the pH, osmolarlity or level of chemical inductants, such as alkanoic acids or their salts.

[0009] Cell cultures of the present invention may optionally be supplemented with nutrients and/or other medium components including hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source. In certain embodiments of the present invention, it may be beneficial to supplement the media with chemical inductants such as hexamethylene-bis(acetamide) ("HMBA") and sodium butyrate ("NaB"). These optional supplements may be added at the beginning of the culture or may be added at a later point in order to replenish depleted nutrients or for another reason. In general, it is desirable to select the initial medium composition to minimize supplementation in accordance with the present invention.

[0010] Various culture conditions may be monitored in accordance with the present invention, including pH, cell density, cell viability, lactate levels, ammonium levels, osmolarity, or titer of the expressed polypeptide or protein.

Exemplary or Independent Claim(s):

1. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a medium characteristic selected from the group consisting of: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (v) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
2. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
3. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative asparagine ratio of less than about 2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
4. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.
5. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1; and

said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

6. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM; and
said medium containing glutamine; and
said medium having two medium characteristics selected from the group consisting of: (i) a medium containing a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and combinations thereof;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce to a viable cell density within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

24. The method of claims 18-23, wherein said measurements are taken daily.

70. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least two medium characteristics selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

71. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least three medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

72. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine and having at least four medium characteristic selected from the group consisting of: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

73. A method of producing a polypeptide in a large-scale production cell culture comprising steps of;
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a defined medium containing glutamine, characterized by: i) a starting amino acid concentration greater than about 70 mM, ii) a molar glutamine to asparagine ratio of less than about 2, iii) a molar glutamine to total amino acid ratio of less than about 0.2, iv) a molar inorganic ion to total amino acid ratio between about 0.4 to 1, and v) a combined glutamine and asparagine concentration greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;

maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that the polypeptide accumulates in the cell culture.

74. A method of producing a polypeptide in a large-scale production cell culture comprising the steps of:
providing a cell culture comprising;
mammalian cells that contain a gene encoding a polypeptide of interest, which gene is expressed under condition of cell culture; and
a medium containing glutamine and having a combined cumulative amount of glutamine and asparagine per unit volume of greater than about 16 mM;
maintaining said culture in an initial growth phase under a first set of culture conditions for a first period of time sufficient to allow said cells to reproduce within a range of about 20%-80% of the maximal possible viable cell density if said culture were maintained under the first set of culture conditions;
changing at least one of the culture conditions, so that a second set of culture conditions is applied;
maintaining said culture for a second period of time under the second set of conditions and for a second period of time so that that the polypeptide accumulates in the cell culture.

76. The method of any one of claims 1-6 or 70-75, wherein:
lactate levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic;
ammonium levels are lower than those levels observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic; and
total amount of produced polypeptide is at least as high as that observed under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.

111-114. (canceled)

Non-exemplary or Dependent Claim(s):

7. The method of claim 1, wherein said cell culture condition in said changing at least one of the culture conditions step is selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.
8. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 13 mM.
9. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 10 mM.
10. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 7 mM.
11. The method of claim 1, wherein the initial glutamine concentration of said medium is less than or equal to 4 mM.
12. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 13 mM.
13. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 10 mM.
14. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 7 mM.
15. The method of claim 1, wherein the total cumulative amount per unit volume of glutamine of said medium is less than or equal to 4 mM.
16. The method of claim 1, wherein glutamine is only provided in the initial medium at the beginning of the cell culture.
17. The method of claim 1, wherein the concentration of soluble iron in the media is greater than 5 [small mu, Greek]M.
18. The method of claim 1, wherein viable cell density of said culture is measured on a periodic basis.
19. The method of claim 1, wherein viability of said culture is measured on a periodic basis.
20. The method of claim 1, wherein said lactate levels of said culture is measured on a periodic basis.
21. The method of claim 1, wherein said ammonium levels of said culture is measured on a periodic basis.
22. The method of claim 1, wherein said titer of said culture is measured on a periodic basis.
23. The method of claim 1, wherein osmolarity of said culture is measured on a periodic basis.
25. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]2 cells/mL.
26. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]3 cells/mL.
27. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]4 cells/mL.
28. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]5 cells/mL.
29. The method of claim 1, wherein the initial density of said mammalian cells is at least 2X10[sup]6 cells/mL.
30. The method of claim 1, wherein the initial density of said mammalian cells is at least 5X10[sup]6 cells/mL.
31. The method of claim 1, wherein the initial density of said mammalian cells is at least 10X10[sup]6 cells/mL.
32. The method of claim 1, wherein the step of providing comprises providing at least about 1000 L of a culture.
33. The method of claim 1, wherein the step of providing comprises providing at least about 2500 L of a culture.
34. The method of claim 1, wherein the step of providing comprises providing at least about 5000 L of a culture.
35. The method of claim 1, wherein the step of providing comprises providing at least about 8000 L of a culture.
36. The method of claim 1, wherein the step of providing comprises providing at least about 10,000 L of a culture.
37. The method of claim 1, wherein the step of providing comprises providing at least about 12,000 L of a culture.
38. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 30 to 42 degrees Celsius.
39. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 32 to 40 degrees Celsius.
40. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 34 to 38 degrees Celsius.
41. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 36 to 37 degrees Celsius.
42. The method of claim 1, wherein said first set of conditions comprises a first temperature range that is approximately 37 degrees Celsius.
43. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 25 to 41 degrees Celsius.
44. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 27 to 38 degrees Celsius.
45. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 35 degrees Celsius.
46. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 29 to 33 degrees Celsius.
47. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 30 to 32 degrees Celsius.
48. The method of claim 1, wherein said second set of conditions comprises a second temperature range that is approximately 31 degrees Celsius.
49. The method of claim 1, further comprising a second changing step subsequent to first said changing at least one of the culture conditions comprising changing at least one of the culture conditions, so that a third set of conditions is applied to the culture.
50. The method of claim 49, wherein the second changing step comprises changing at least one culture condition selected from the group consisting of: (i) temperature, (ii) pH, (iii) osmolality, (iv) chemical inductant level, and combinations thereof.
51. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 25 to 40

degrees Celsius.

52. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 27 to 37 degrees Celsius.
53. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 29 to 34 degrees Celsius.
54. The method of claim 49, wherein said third set of conditions comprises a third temperature range that is approximately 30 to 32 degrees Celsius.
55. The method of claim 1, wherein said first period of time is between 0-8 days.
56. The method of claim 1, wherein said first period of time is between 1-7 days.
57. The method of claim 1, wherein said first period of time is between 2-6 days.
58. The method of claim 1, wherein said first period of time is between 3-5 days.
59. The method of claim 1, wherein said first period of time is approximately 4 days.
60. The method of claim 1, wherein said first period of time is approximately 5 days.
61. The method of claim 1, wherein said first period of time is approximately 6 days.
62. The method of claim 1, wherein the total of said first period of time and said second period of time is at least 5 days.
63. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the lactate level decreases subsequent to the lactate level in the culture reaching a maximal level.
64. The method of claim 1, wherein in the step of maintaining said culture for a second period of time, the ammonium level decreases subsequent to the ammonium level in the culture reaching a maximal level.
65. The method of claim 1, wherein said total amount of said produced polypeptide is at least 1.5-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
66. The method of claim 1, wherein said total amount of said produced polypeptide is at least 2-fold higher that the amount of polypeptide produced under otherwise identical conditions in otherwise identical medium that lacks said medium characteristic.
67. The method of claim 1, wherein said cell culture is further provided with supplementary components.
68. The method of claim 67, wherein said supplementary components are provided at multiple intervals.
69. The method of claim 67 wherein said supplementary components are selected from a group consisting of hormones and/or other growth factors, particular ions (such as sodium, chloride, calcium, magnesium, and phosphate), buffers, vitamins, nucleosides or nucleotides, trace elements (inorganic compounds usually present at very low final concentrations), amino acids, lipids, or glucose or other energy source.
75. The method of claim 1, wherein said medium comprises a medium containing glutamine and having a medium characteristic selected from the group consisting of:
(i) a starting amino acid concentration greater than about 70 mM,
(ii) a molar starting glutamine to starting asparagine ratio of less than about 2, (iii) a molar starting glutamine to starting total amino acid ratio of less than about 0.2, (iv) a molar starting inorganic ion to starting total amino acid ratio between about 0.4 to 1, (v) a combined starting glutamine and starting asparagine concentration greater than about 16 mM, and combinations thereof.
77. The method of claim 1, wherein said culture is not supplemented with additional components over the course of producing said polypeptide.
78. The method of claim 1, wherein said culture is not supplemented with additional glutamine over the course of producing said polypeptide.
79. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted prior to said step of changing to a second set of culture conditions.
80. The method of claim 1, wherein the glutamine concentration in said culture is substantially depleted at approximately the same time as said step of changing to a second set of culture conditions.
81. The method of claim 1, wherein glycylglutamine is substituted for glutamine in said culture.
82. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative asparagine ratio of less than about 2, (iii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iv) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (v) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 mM.
83. The method of claim 1, wherein said medium contains: (i) a cumulative amino acid amount per unit volume greater than about 70 mM, (ii) a molar cumulative glutamine to cumulative total amino acid ratio of less than about 0.2, (iii) a molar cumulative inorganic ion to cumulative total amino acid ratio between about 0.4 to 1, and (iv) a combined cumulative amount of glutamine and asparagine per unit volume greater than about 16 a mM.
84. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
85. The method of claim 1, wherein the cumulative total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.
86. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 25 mM.
87. The method of claim 1, wherein the initial total amount of histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine, and proline per unit volume in said medium is greater than approximately 35 mM.
88. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:
(i) a cumulative total amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) a cumulative total amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) a cumulative total amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) a cumulative total amount of methionine per unit volume greater than approximately 2.0 mM;
(v) a cumulative total amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM;
(vii) a cumulative total amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) a cumulative total amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) a cumulative total amount of proline per unit volume greater than approximately 2.5 mM.
89. The method of claim 1, wherein said medium has a medium characteristic selected from the group consisting of:
(i) an initial amount of histidine per unit volume greater than approximately 1.7 mM;
(ii) an initial amount of isoleucine per unit volume greater than approximately 3.5 mM;
(iii) an initial amount of leucine per unit volume greater than approximately 5.5 mM;
(iv) an initial amount of methionine per unit volume greater than approximately 2.0 mM;
(v) an initial amount of phenylalanine per unit volume greater than approximately 2.5 mM;
(vi) an initial amount of proline per unit volume reater than approximately 2.5 mM;

(vii) an initial amount of tryptophan per unit volume greater than approximately 1.0 mM;
(viii) an initial amount of tyrosine per unit volume greater than approximately 2.0 mM; and
(ix) an initial amount of proline per unit volume greater than approximately 2.5 mM.

90. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 7 mM.
91. The method of claim 1, wherein the cumulative total amount of serine per unit volume in said medium is greater than approximately 10 mM.
92. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.
93. The method of claim 1, wherein the cumulative total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
94. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 8 mM.
95. The method of claim 1, wherein the initial total amount of asparagine per unit volume in said medium is greater than approximately 12 mM.
96. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 2.5 mM.
97. The method of claim 1, wherein the cumulative total amount of phosphorous per unit volume in said medium is greater than approximately 5 mM.
98. The method of claim 1, wherein the cumulative total amount of glutamate per unit volume in said medium is less than approximately 1 mM.
99. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 8 mg/L.
100. The method of claim 1, wherein the cumulative total amount of calcium pantothenate per unit volume in said medium is greater than approximately 20 mg/L.
101. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 7 mg/L.
102. The method of claim 1, wherein the cumulative total amount of nicotinamide per unit volume in said medium is greater than approximately 25 mg/L.
103. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 5 mg/L.
104. The method of claim 1, wherein the cumulative total amount of pyridoxine and pyridoxal per unit volume in said medium is greater than approximately 35 mg/L.
105. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 1.0 mg/L.
106. The method of claim 1, wherein the cumulative total amount of riboflavin per unit volume in said medium is greater than approximately 2.0 mg/L.
107. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 7 mg/L.
108. The method of claim 1, wherein the cumulative total amount of thiamine hydrochloride per unit volume in said medium is greater than approximately 35 mg/L.
109. The method of claim 1, wherein the polypeptide is anti-%GDF%-8.
110. The method of claim 1, wherein the polypeptide is anti-LewY.

6437678
Derwent Accession: 2006-172088

UTILITY

Combination therapy for diabetes, obesity and cardiovascular diseases using %GDF%-8 inhibitors

Inventor: Tobin, James F., Newton, MA, US
Assignee: Wyeth, (02)
Correspondence Address: FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER;LLI
901 NEW YORK AVENUE, NW, WASHINGTON, DC, 20001-4413, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20060034831	A1	20060216	US 2005201825	20050811
Provisional				US 60-600784	20040812

US Classification on document (Main): 424130100
(X-ref): 514003000; 514369000; 514423000; 514635000; 514592000

International Classification (v8 + Attributes)
IPC + Level Value Position Status Version Action Source Office:
A61K-0039/395 A I F B 20060101 20060216 H US
A61K-0038/28 A I L B 20060101 20060216 H US
A61K-0031/426 A I L B 20060101 20060216 H US
A61K-0031/401 A I L B 20060101 20060216 H US
A61K-0031/175 A I L B 20060101 20060216 H US
A61K-0031/155 A I L B 20060101 20060216 H US

Fulltext Word Count: 20902
Number of Claims: 35
Exemplary or Independent Claim Number(s): 1,21

References to Related Applications:

RELATED CASES

[0001] This application claims the benefit of U.S. Provisional Application No. 60/600,784, filed Aug. 12, 2004, the contents of which are incorporated herein in their entirety by reference.

Abstract:

[00000] A method of treating obesity, cardiovascular diseases, and disorders of insulin metabolism in a subject, comprising administering to the subject a therapeutically effective amount of a %GDF%-8 inhibitor, and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

Summary of the Invention:

TECHNICAL FIELD

[0002] This invention relates to methods of treating at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism, such as diabetes and syndrome X, using combination therapy. The novel combination therapy employs at least one inhibitor of growth and differentiation factor-8 (%GDF%-8) and at least one other therapeutic agent.

BACKGROUND OF THE INVENTION

[0003] Growth and differentiation factor-8 (%GDF%-8), also known as myostatin, is a secreted protein and is a member of the transforming growth factor-beta (TGF-[small beta, Greek]) superfamily of structurally related growth factors, all of which possess physiologically important growth-regulatory and morphogenetic properties (Kingsley et al., Genes Dev. 8:133-146 (1994); Hoodless et al., Curr. Topics Microbiol. Immunol. 228:235-272 (1998)). Similarly to TGF-[small beta, Greek], human %GDF%-8 is synthesized as a 375 amino acid long precursor protein. The precursor %GDF%-8 protein forms a homodimer. During processing the amino-terminal propeptide is cleaved off at Arg-266. The cleaved propeptide, known as the "latency-associated peptide" (LAP), may remain noncovalently bound to the homodimer, thereby inactivating the complex (Miyazono et al., J. Biol. Chem. 263:6407-6415 (1988); Wakefield et al., J. Biol. Chem. 263:7646-7654 (1988); Brown et al., Growth Factors 3:35-43 (1990); and Thies et al., Growth Factors 18:251-259 (2001)). The complex of mature %GDF%-8 with propeptide is commonly referred to as the "small latent complex" (Gentry et al., Biochemistry 29:6851-6857 (1990); Derynck et

al., Nature, 316:701-705 (1995); and Massague, Ann. Rev. Cell Biol. 12:597-641 (1990)). Other proteins are also known to bind to mature %GDF%-8 and inhibit its biological activity. Such inhibitory proteins include follistatin and follistatin-related proteins (Gamer et al., Dev. Biol., 208:222-232 (1999)).

[0004] An alignment of deduced amino acid sequences from various species demonstrates that %GDF%-8 is highly conserved throughout evolution (McPherron et al., Proc. Nat. Acad. Sci. U.S.A. 94:12457-12461 (1997)). In fact, the sequences of human, mouse, rat, porcine, and chicken %GDF%-8 are 100% identical in the C-terminal region, while those of baboon, bovine, and ovine differ by 3 amino acids or less. The zebrafish %GDF%-8 is the most diverged; however, it is still 88% identical to human.

[0005] The high degree of conservation suggests that %GDF%-8 has an essential function. %GDF%-8 is highly expressed in the developing and adult skeletal muscle and was found to be involved in the regulation of critical biological processes in the muscle and in osteogenesis. For example, %GDF%-8 knockout transgenic mice are characterized by a marked hypertrophy and hyperplasia of the skeletal muscle (McPherron et al., Nature 387:83-90 (1997)) and altered cortical bone structure (Hamrick et al., Bone 27:343-349 (2000)). Similarly, increases in skeletal muscle mass are evident in naturally occurring mutations of %GDF%-8 in cattle (Ashmore et al., Growth, 38:501-507 (1974); Swatland et al., J. Anim. Sci. 38:752-757 (1994); McPherron et al., Proc. Nat. Acad. Sci. U.S.A. 94:12457-12461 (1997); and Kambadur et al., Genome Res. 7:910-915 (1997)). Studies have indicated that muscle wasting associated with HIV-infection is accompanied by an increase in %GDF%-8 expression (Gonzalez-Cadavid et al., Proc. Nat. Acad. Sci. U.S.A. 95:14938-14943 (1998)). %GDF%-8 has also been implicated in the production of muscle-specific enzymes (e.g., creatine kinase) and proliferation of myoblast cells (WO 00/43781). In addition to its growth-regulatory and morphogenetic properties, %GDF%-8 is thought to be also involved in a number of other physiological processes, including glucose homeostasis in the development of type 2 diabetes, impaired glucose tolerance, metabolic syndromes (e.g., syndrome X), insulin resistance induced by trauma, such as burns or nitrogen imbalance, and adipose tissue disorders (e.g., obesity) (Kim et al. BBRC 281:902-906 (2001)).

[0006] Other studies extend the role of %GDF%-8 in adipogenesis and glucose homeostasis. For example, injection of %GDF%-8 secreting tumor cells into mice increases their level of blood sugar (hyperglycemia) and decreases their weight and muscle mass. Also %GDF%-8 blocks insulin-induced expression of GLUT4, and it blocks insulin-mediated differentiation of pre-adipocytes. Collectively, the %GDF%-8 studies suggest that inhibition of %GDF%-8 would reduce blood sugar and body fat, and increase insulin-mediated transport of glucose, conditions that may benefit a patient having or who may ultimately acquire type 2 diabetes or syndrome X, or other syndromes involving glucose homeostasis.

[0007] Obesity, cardiovascular diseases, and/or disorders of insulin metabolism, such as diabetes and/or syndrome X have been treated using a number of different therapies. These therapies include angiotensin converting enzyme inhibitors, sulfonylurea agents, antilipemic agents, biguanide agents, thiazolidinedione agents, insulin, alpha-glucosidase inhibitors, and aldose reductase inhibitors, although not all the therapies have been recognized for the treatment of all the diseases and disorders described. These therapies work through a variety of mechanisms, none of which are related to %GDF%-8.

SUMMARY OF THE INVENTION

[0008] The present invention relates to methods of treating at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism, including diabetes and syndrome X, by administering an effective amount a %GDF%-8 inhibitor in combination with at least one other therapeutic agent.

[0009] At least one of obesity, cardiovascular diseases, and disorders of insulin metabolism, such as diabetes and syndrome X, may be treated with inhibitors of %GDF%-8 in combination with other therapeutic agents that treat these targeted syndromes. This approach to treatment is called combination therapy. A variety of other therapeutics have been used to treat the different causes and diseases associated these targeted syndromes, including agents to stimulate glucose transport (e.g., insulin, sulfonylurea agents, biguanide agents, thiazolidinedione agents), agents to control blood sugar (e.g., alpha-glucosidase inhibitors), agents to improve cardiovascular health (e.g., antilipemic agents and ACE inhibitors), and agents to reduce toxic sorbitol production in the eye and nerves (e.g., aldose reductase inhibitors). It is accordingly a primary object of this invention to provide an improved treatment in the form of a combination therapy, for at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism, such as diabetes and syndrome X, using %GDF%-8 inhibitors in combination with at least one other therapeutic agent that treats the targeted syndromes.

[0010] One object of this invention is to create a method of treating at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism in a subject, comprising administering to the subject a therapeutically effective amount of a %GDF%-8 inhibitor, and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

[0011] A further object of this invention is to create a pharmaceutical composition for treating at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism in a subject, comprising administering to the subject a therapeutically effective amount of a %GDF%-8 inhibitor, and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

[0012] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0013] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

[0014] The accompanying drawings, which are incorporated in and constitute a part of this specification, and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE SEQUENCES

[0015]

(Table 1. See patent image)

[0016]

(Table 2. See patent image)

Description of the Sequences:
(Sequence listing SEQLST-1 - see patent image)

Exemplary or Independent Claim(s):

1. A method of treating a targeted syndrome in a subject, comprising administering to the subject a therapeutically effective amount of at least one %GDF%-8 inhibitor, and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

21. A pharmaceutical composition useful for treating a targeted syndrome comprising combining a therapeutically effective amount of a %GDF%-8 inhibitor and a therapeutically effective amount of at least one other therapeutic agent which treats the targeted syndrome.

Non-exemplary or Dependent Claim(s):

2. A method according to claim 1, wherein the targeted syndrome is chosen from at least one of obesity, cardiovascular diseases, and disorders of insulin metabolism.
3. A method according to claim 1, wherein the %GDF%-8 inhibitor is chosen from at least one of an antibody against %GDF%-8, an antibody against %GDF%-8 receptor, a modified soluble receptor, a protein binding to %GDF%-8, a protein binding to %GDF%-8 receptor, inhibitors of protease activation of the %GDF%-8 small latent complex, and %GDF% -8 inhibiting mimetics thereof.
4. A method according to claim 3, wherein the %GDF%-8 inhibitor specifically binds a mature %GDF%-8 protein.
5. The method according to claim 1, wherein the therapeutic agent is chosen from at least one of an angiotensin converting enzyme (ACE) inhibitor, a sulfonylurea agent, an antilipemic agent, a biguanide agent, a thiazolidinedione agent, insulin, an alpha-glucosidase inhibitor, an aldose reductase inhibitor, or a PTPase inhibitor.
6. The method of claim 5, wherein the angiotensin converting enzyme (ACE) inhibitor is chosen from at least one of quinapril, ramipril, verapamil, captopril, diltiazem, clonidine, hydrochlorthiazide, benazepril, prazosin, fosinopril, lisinopril, atenolol, enalapril, perindropril, perindropril tert-butylamine, trandolapril and moexipril, and the suitable pharmaceutically acceptable salt forms thereof.
7. The method of claim 5, wherein the sulfonylurea agent is chosen from at least one of glipizide, glyburide (glibenclamide), chlorpropamide, tolbutamide, tolazamide and glimepriride, and the pharmaceutically acceptable salt forms thereof.
8. The method of claim 5, wherein the antilipemic agent is chosen from at least one of bile acid sequestrants, fibric acid derivatives, HMG-CoA reductase inhibitors and nicotinic acid compounds, and the pharmaceutically acceptable salt forms thereof.
9. The method of claim 5, wherein the biguanide agent is chosen from at least one of metformin and its pharmaceutically acceptable salt forms.
10. The method of claim 5, wherein the thiazolidinedione agent is chosen from at least one of pioglitazone and rosiglitazone, and the pharmaceutically acceptable salt forms thereof.
11. The method of claim 5, wherein the insulin is chosen from at least one of rapid acting insulins, intermediate acting insulins, long acting insulins and combinations of intermediate and rapid acting insulins.
12. The method of claim 5, wherein the alpha-glucosidase inhibitor is chosen from at least one of miglitol and acarbose, and the pharmaceutically acceptable salt forms thereof.
13. The method of claim 5, wherein the aldose reductase inhibitor is chosen from at least one of:
    a) a spiro-isoquinoline-pyrrolidine tetrone compound;
    b) 2-[(4-bromo-2-fluorophenyl)methyl]-6-fluoro-(9Cl);
    c) Tolrestat,
    d) Sorbinil;
    e) Methosorbinil;
    f) Zopolrestat;
    g) Epalrestat;
    h) Zenarestat;
    i) Imirestat;
    j) Ponalrestat;
    k) ONO-2235;
    l) GP-1447;
    m) CT-112;
    n) BAL-ARI 8;
    o) AD-5467;
    p) ZD5522;
    q) 3,4-dihydro-2,8-diisopropyl-3-thioxo-2H-1,4-benzoxazine-4-acetic acid;
    r) 1-[(3-bromo-2-benzofuranyl)sulfonyl]-2,4-imidazolidinedione (M-16209): NZ-314, which is 1-Imidazolidineacetic acid, 3-[(3-nitrophenyl)methyl]-2,4,5-trioxo-(9Cl);
    s) 1-phthalazineacetic acid, 3,4-dihydro-4-oxo-3-[[5-trifluoromethyl)-2-benzothiazolyl]methyl]-;
    t) M-79175;
    u) SPR-210;
    v) Spiro[pyrrolidine-3,6'(5'H)-pyrrolo[1,2,3-de][1,4]benzoxazine]-2,5,5' -trione, 8'-chloro-2',3'-dihydro-(9Cl);
    w) 6-fluoro-2,3-dihyro-2',5'-dioxo-(2S-cis)-spiro[4H-l-benzopyran-4,4'-i midazolidine]-2-carboxyamide; and
    x) analogs and pharmaceutically acceptable salts thereof.
14. The method of claim 5, wherein the PTPase inhibitor is chosen from at least one compound with the formula (I):

(Chemical formulae 5. See patent image)

R[sub]1 is C(O)OR[sub]7, 5- to 6-membered heterocycle, H, halogen, CN, or C(O)NR[sub]7R[sub]8;
R[sub]2 is C(O)ZR[sub]4 or CN;
Z is -O- or -NR[sub]5-;
X is -O-C[sub]1-3alkylene-, -NR[sub]8-C[sub]1-3alkylene-, -S-C[sub]1-3alkylene-, -SO-C[sub]1-3alkylene-, -SO[sub]2-C[sub]1-3alkylene-, -C[sub]1-4alkylene-, -C[sub]2-4alkenylene-, or -C[sub]2-4alkynylene-, wherein any of the alkylene, alkenylene and alkynylene groups can be optionally substituted with one or more halogen, oxo, HN[horizontal line], CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, R[sub]4, or Q;
each Y[sub]1, Y[sub]2, Y[sub]3, Y[sub]4, and Y[sub]5 is, independently, CR[sub]3, N, S, or O, one or two of Y[sub]1, Y[sub]2, Y[sub]3, Y[sub]4, and Y[sub]5 can be absent;
each R[sub]3 is, independently, H, aryl, 5- to 8-membered heterocyclyl, C[sub]1-6alkyl, C[sub]2-6alkenyl, C[sub]2-6alkynyl, halogen, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, or Q, wherein any of the aryl, heterocyclic, alkyl, alkenyl or alkynyl groups is optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, N[sub]3, R[sub]4, or Q;
each Q is, independently, -OC(O)NR[sub]4R[sub]5, -OR[sub]4, -OC(O)R[sub]4, -COOR[sub]4, -C(O)NR[sub]4R[sub]5, -C(O)R[sub]4, -C([horizontal line]N-OH)R[sub]4, -NR[sub]4R[sub]5, -N[sup]+R[sub]4R[sub]5R[sub]6, -NR[sub]4C(O)R[sub]5, -NR[sub]4C(O)NR[sub]5R[sub]6, -NR[sub]4C(O)OR[sub]5, -NR[sub]4S(O)[sub]2R[sub]5, -SR[sub]4, -S(O)R[sub]4, -S(O)[sub]2R[sub]4, or -S(O)[sub]2NR[sub]4R[sub]5;
each R[sub]4, R[sub]5, and R[sub]6 is, independently, H, C[sub]1-16alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-8cycloalkyl, cycloalkylC[sub]1-6alkyl, 5- to 8-membered heterocycle, heterocyclicC[sub]1-6alkyl, aryl, arylC[sub]1-6alkyl, arylC[sub]2-6alkenyl, or arylC[sub]2-6alkynyl, each R[sub]4, R[sub]5, and R[sub]6 can be optionally substituted with one or more C[sub]1-6alkyl, C[sub]2-6alkenyl, C[sub]2-6alkynyl, halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, N[sub]3, -OC(O)NR[sub]7R[sub]8, -OR[sub]7, -OC(O)R[sub]7, -COOR[sub]7, -C(O)NR[sub]7R[sub]8, -C(O)R[sub]7, -NR[sub]7R[sub]8, -N[sup]+R[sub]7R[sub]8R[sub]9, -NR[sub]7C(O)R[sub]8, -NR[sub]7C(O)NR[sub]8R[sub]9, -NR[sub]7C(O)OR[sub]8, -NR[sub]7S(O)[sub]2R[sub]8, -SR[sub]7, -S(O)R[sub]7, -S(O)[sub]2R[sub]7, or -S(O)[sub]2NR[sub]7R[sub]8; each R[sub]7, R[sub]8, and R[sub]9 is, independently, H, C[sub]1-12alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-12cycloalkyl, aryl, or arylC[sub]1-12alkyl, each R[sub]7, R[sub]8, and R[sub]9 can be optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, or NO[sub]2;
when the ring system is 1-benzothiophene, R[sub]1 is C(O)OCH[sub]3, and X is -OCH[sub]2-, then R[sub]2 is not C(O)OCH[sub]3;
when the ring system is 1-benzothiophene, R[sub]1 is C(O)OH, and X is -OCH[sub]2-, then R[sub]2 is not C(O)OH;
when the ring system is thieno[2,3-b]pyridine, R[sub]1 is isopropyl ester, and X is -OCH[sub]2-, then R[sub]2 is not C[sub]1-3alkyl ester;
when the ring system is thieno[2,3-b]pyridine, R, is C(O)OC[sub]1-4alkyl, and X is -OCH[sub]2- or -OCH(CH[sub]3)-, then R[sub]2 is not CN;
when the ring system is thieno[2,3-b]pyridine, R, is isopropyl ester, and X is -SCH[sub]2CH[sub]2-, then R[sub]2 is not CN; and
when the ring system is thieno[2,3-b]pyridine, R[sub]1 is isopropyl

ester, and X is -SCH[sub]2-, then R[sub]2 is not isopropyl ester.

15. The method of claim 5, wherein the PTPase inhibitor is chosen from at least one compound with the formula (II):

(Chemical formulae 6. See patent image)

R[sub]1 is R[sub]5, OR[sub]5, C(O)OR[sub]5, C(O)R[sub]5, or C(O)NR[sub]5R[sub]6;
R[sub]2 is R[sub]5;
X is -O-C[sub]1-3alkylene-, -NR[sub]8-C[sub]1-3alkylene-, -S-C[sub]1-3alkylene-, -SO-C[sub]1-3alkylene-, -SO[sub]2-C[sub]1-3alkylene-, -C[sub]1-4alkylene-, -C[sub]2-4alkenylene-, or -C[sub]2-4alkynylene-, wherein any of the alkylene, alkenylene or alkynylene groups can be optionally substituted with one or more halogen, oxo, imido, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, or Q;
Y is absent, -O-, or -NR[sub]6-;
R[sub]3 is H, halogen, CN, CF[sub]3, OCF[sub]3, C[sub]1-3 alkyl, C[sub]3-4cycloalkyl, C[sub]1-3alkoxy, or aryl;
R[sub]4 is A-B-E-D, where A is absent or arylene, heteroarylene, C[sub]1-6alkylene, C[sub]2-6 alkenyldiyl, or C[sub]2-6alkynyl, each A can be optionally substituted with one or more of C[sub]1-6alkyl, C[sub]2-6alkenyl, C[sub]2-6alkynyl, halogen, CN, OCF[sub]3, OH, NH[sub]2, CHO, NO[sub]2, or Q, any of the alkyl, alkenyl or alkynyl groups is optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, N[sub]3, or Q;
each A can be optionally terminated with one or more arylene, alkylene, or alkenylene;
B is absent or -NR[sub]5-, -NR[sub]7-, -N(R[sub]5)CH[sub]2-, -N(R[sub]7)CH[sub]2-, -N(R[sub]9)-, -N(R[sub]9)C(O)-, -N(R[sub]9)C(O)C(R[sub]11)(R[sub]12)-, -N(R[sub]9)C(O)C(O)-, -N(R[sub]9)C(O)N(R[sub]10)-, -N(R[sub]9)SO[sub]2-, -N(R[sub]9)SO[sub]2C(R[sub]10)(R[sub]11)-, -N(R[sub]9)(R[sub]10)C(R[sub]11)(R[sub]12)-, -N(R[sub]9)C(R[sub]11)(R[sub]12)C(R[sub]13)(R[sub]14)-, -O-, -O-C(R[sub]11)(R[sub]12)-, -O-C(R[sub]11)(R[sub]12)C(R[sub]13)(R[sub]14)-, -C(R[sub]11)(R[sub]12)-O-, -C(R[sub]11)(R[sub]12)-O-C(R[sub]13)(R[sub]14)-, -C(R[sub]11)(R[sub]12)N(R[sub]9)-, -C(R[sub]11)(R[sub]12)N(R[sub]9)C(R[sub]13)(R[sub]14)-, -C(R[sub]11)(R[sub]12)S-, -C(R[sub]11)(R[sub]12)SC(R[sub]13)(R[sub]14)-, or -C(R[sub]11)(R[sub]12)SO[sub]2C(R[sub]13)(R[sub]14)-;
E is absent or C[sub]3-12cycloalkylene, 3- to 12-membered heterocycdiyl, arylene, C[sub]1-12alkylene, C[sub]2-12alkenylene, or C[sub]2-12alkynylene, where each E is optionally substituted with one or more C[sub]1-3alkyl, C[sub]1-3alkoxy, halogen, CN, OH, NH[sub]2, or NO[sub]2;
D is one or more H, halogen, OH, NH[sub]2, CHO, CN, NO[sub]2, CF[sub]3, or Q;
when A, B, and E are absent, R[sub]1 is C(O)OH or C(O)OCH[sub]3, R[sub]2 is H, and R[sub]3 is H or chlorine, D is not H or chlorine;
and when A, B, and E are absent, R[sub]1 is C(O)OH or C(O)OCH[sub]3, R[sub]2 is H, and R[sub]3 is H or bromine, D is not H or bromine;
each Q, independently, is -R[sub]5, -R[sub]7, -OR[sub]5, -OR[sub]7, -NR[sub]5R[sub]6, -NR[sub]5R[sub]7, -N[sup]+R[sub]5R[sub]6R[sub]8, S(O)[sub]nR[sub]5, or -S(O)[sub]nR[sub]7, and n is 0, 1, or 2;
each R[sub]5, R[sub]5, and R[sub]8, independently, is H, C[sub]1-12alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-12cycloalkyl. C[sub]1-12alkoxyC[sub]1-12alkyl, cycloalkylC[sub]1-6alkyl, 3- to 8-membered heterocycyl, heterocycylC[sub]1-6alkyl, aryl, arylC[sub]1-6 alkyl, arylC[sub]2-6 alkenyl, or arylC[sub]2-6 alkynyl, each R[sub]5, R[sub]6, and R[sub]8 can be optionally substituted with one or more R[sub]9, -OR[sub]9, -OC(O)OR[sub]9, -C(O)R[sub]9, -C(O)OR[sub]9, -C(O)NR[sub]9R[sub]10, -SR[sub]9, -S(O)R[sub]9, -S(O)[sub]2R[sub]9, -NR[sub]9R[sub]10, -N[sup]+R[sub]9R[sub]10R[sub]11, -NR[sub]9C(O)R[sub]10, -NC(O)NR[sub]9R[sub]10, -NR[sub]9S(O)[sub]2R[sub]10, oxo, halogen, CN, OCF[sub]3, CF[sub]3, OH, or NO[sub]2;
R[sub]7 is -C(O)R[sub]5, -C(O)OR[sub]5, -C(O)NR[sub]5R[sub]6, -S(O)[sub]2R[sub]5, -S(O)R[sub]5, or -S(O)[sub]2NR[sub]5R[sub]6;
each R[sub]9, R[sub]10 R[sub]11, R[sub]12, R[sub]13 and R[sub]14 is, independently, H, C[sub]1-12alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-12cycloalkyl, aryl, or arylC[sub]1-12alkyl, any of the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or arylalkyl groups is optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, or NO[sub]2.

16. The method of claim 1, wherein the administration is sequential.

17. The method of claim 1, wherein the administration is simultaneous.

18. The method of claim 1, wherein the administration of at least one therapeutic agent is oral.

19. The method of claim 1, wherein the administration is parenteral.

20. The method of claim 19, wherein the parenteral administration is intravenous.

22. A pharmaceutical composition according to claim 21, wherein the %GDF%-8 inhibitor is chosen from at least one of an antibody against %GDF%-8, an antibody against %GDF%-3 receptor, a modified soluble receptor, a protein binding to %GDF%-8, a protein binding to %GDF%-8 receptor, inhibitors of protease activation of the %GDF%-8 small latent complex, and %GDF%-3 inhibiting mimetics thereof.

23. A pharmaceutical composition according to claim 22 wherein the protein binding to %GDF%-8 is chosen from at least one of a %GDF%-8 propeptide having SEQ ID NO:65, a mutated %GDF%-8 propeptide, follistatin, follistatin-domain containing proteins, and Fc fusions thereof.

24. A pharmaceutical composition according to claim 22, wherein the %GDF%-8 inhibitor specifically binds a mature %GDF%-8 protein.

25. The pharmaceutical composition according to claim 21, wherein the therapeutic agent is chosen from at least one of an angiotensin converting enzyme (ACE) inhibitor, a sulfonylurea agent, an antilipemic agent, a biguanide agent, a thiazolidinedione agent, insulin, an alpha-glucosidase inhibitor, an aldose reductase inhibitor, or a PTPase inhibitor.

26. The pharmaceutical composition of claim 25, wherein the angiotensin converting enzyme (ACE) inhibitor is chosen from at least one of quinapril, ramipril, verapamil, captopril, diltiazem, clonidine, hydrochlorthiazide, benazepril, prazosin, fosinopril, lisinopril, atenolol, enalapril, perindropril, perindropril tert-butylamine, trandolapril and moxipril, or a pharmaceutically acceptable salt form of one or more of these compounds.

27. The pharmaceutical composition of claim 25, wherein the sulfonylurea agent is chosen from at least one of glipizide, glyburide (glibenclamide), chlorpropamide, tolbutamide, tolazamide and glimepriride, and the pharmaceutically acceptable salt forms thereof.

28. The pharmaceutical composition of claim 25, wherein the antilipemic agent is chosen from at least one of bile acid sequestrants, fibric acid derivatives, HMG-CoA reductase inhibitors and nicotinic acid compounds, and the pharmaceutically acceptable salt forms thereof.

29. The pharmaceutical composition of claim 25, wherein the biguanide agent is chosen from at least one of metformin and its pharmaceutically acceptable salt forms.

30. The pharmaceutical composition of claim 25, wherein the thiazolidinedione agent is chosen from at least one of pioglitazone and rosiglitazone, and pharmaceutically acceptable salt forms of these agents.

31. The pharmaceutical composition of claim 25, wherein the insulin is chosen from at least one of rapid acting insulins, intermediate acting insulins, long acting insulins and combinations of intermediate and rapid acting insulins.

32. The pharmaceutical composition of claim 25, wherein the alpha-glucosidase inhibitor is chosen from at least one of miglitol and acarbose, and a pharmaceutically acceptable salt form of one or more of these compounds.

33. The pharmaceutical composition of claim 25, wherein the aldose reductase inhibitor is chosen from at least one of
a) a spiro-isoquinoline-pyrrolidine tetrone compound;
b) 2-[(4-bromo-2-fluorophenyl)methyl]-6-fluoro-(9CI);
c) Tolrestat,
d) Sorbinil;
e) Methosorbinil;
f) Zopolrestat;
g) Epalrestat;

h) Zenarestat;
i) Imirestat;
j) Ponalrestat;
k) ONO-2235;
l) GP-1447;
m) CT-112;
n) BAL-ARI 8;
o) AD-5467;
p) ZD5522;
q) 3,4-dihydro-2,8-diisopropyl-3-thioxo-2H-1,4-benzoxazine-4-acetic acid;
r) 1-[(3-bromo-2-benzofuranyl)sulfonyl]-2,4-imidazolidinedione (M-16209): NZ-314, which is 1-Imidazolidineacetic acid, 3-[(3-nitrophenyl)methyl]-2,4,5-trioxo-(9CI);
s) 1-phthalazineacetic acid, 3,4-dihydro-4-oxo-3-[[5-trifluoromethyl)-2-benzothiazolyl]methyl]-;
t) M-79175;
u) SPR-210;
v) Spiro[pyrrolidine-3,6'(5'H)-pyrrolo[1,2,3-de][1,4]benzoxazine]-2,5,5'-trione, 8'-chloro-2',3'-dihydro-(9CI);
w) 6-fluoro-2,3-dihyro-2',5'-dioxo-(2S-cis)-spiro[4H-1-benzopyran-4,4'-imidazolidine]-2-carboxyamide;
analogs and pharmaceutically acceptable salts thereof.

34. The pharmaceutical composition of claim 25, wherein the PTPase inhibitor is chosen from at least one compound with the formula (I):

(Chemical formulae 7. See patent image)

R[sub]1 is C(O)OR[sub]7, 5- to 6-membered heterocycle, H, halogen, CN, or C(O)NR[sub]7R[sub]8;
R[sub]2 is C(O)ZR[sub]4 or CN;
Z is -O- or -NR[sub]5-;
X is -O-C[sub]1-3alkylene-, -NR[sub]8-C[sub]1-3alkylene-, -S-C[sub]1-3alkylene-, -SO-C[sub]1-3alkylene-, -SO[sub]2-C[sub]1-3alkylene-, -C[sub]1-4alkylene-, -C[sub]2-4alkenylene-, or -C[sub]2-4alkynylene-, wherein any of the alkylene, alkenylene and alkynylene groups can be optionally substituted with one or more halogen, oxo, HN[horizontal line], CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, R[sub]4, or Q;
each Y[sub]1, Y[sub]2, Y[sub]3, Y[sub]4, and Y[sub]5 is, independently, CR[sub]3, N, S, or O, one or two of Y[sub]1, Y[sub]2, Y[sub]3, Y[sub]4, and Y[sub]5 can be absent;
each R[sub]3 is, independently, H, aryl, 5- to 8-membered heterocyclyl, C[sub]1-6alkyl, C[sub]2-6alkenyl, C[sub]2-6alkynyl, halogen, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, or Q, wherein any of the aryl, heterocyclic, alkyl, alkenyl or alkynyl groups is optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, N[sub]3, R[sub]4, or Q;
each Q is, independently, -OC(O)NR[sub]4R[sub]5, -OR[sub]4, -OC(O)R[sub]4, -COOR[sub]4, -C(O)NR[sub]4R[sub]5, -C(O)R[sub]4, -C([horizontal line]N-OH)R[sub]4, -NR[sub]4R[sub]5, -N[sup]+R[sub]4R[sub]5R[sub]6, -NR[sub]4C(O)R[sub]5, -NR[sub]4C(O)NR[sub]5R[sub]6, -NR[sub]4C(O)OR[sub]5, -NR[sub]4S(O)[sub]2R[sub]5, -SR[sub]4, -S(O)R[sub]4, -S(O)[sub]2R[sub]4, or -S(O)[sub]2NR[sub]4R[sub]5;
each R[sub]4, R[sub]5, and R[sub]6 is, independently, H, C[sub]1-16alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-8cycloalkyl, cycloalkylC[sub]1-6alkyl, 5- to 8-membered heterocycle, heterocyclicC[sub]1-6alkyl, aryl, arylC[sub]1-6alkyl, arylC[sub]2-6alkenyl, or arylC[sub]2-6alkynyl, each R[sub]4, R[sub]5, and R[sub]6 can be optionally substituted with one or more C[sub]1-6alkyl, C[sub]2-6alkenyl, C[sub]2-6alkynyl, halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, N[sub]3, -OC(O)NR[sub]7R[sub]8, -OR[sub]7, -OC(O)R[sub]7, -COOR[sub]7, -C(O)NR[sub]7R[sub]8, -C(O)R[sub]7, -NR[sub]7R[sub]8, -N[sup]+R[sub]7R[sub]8R[sub]9, -NR[sub]7C(O)R[sub]8, -NR[sub]7C(O)NR[sub]8R[sub]9, -NR[sub]7C(O)OR[sub]8, -NR[sub]7S(O)[sub]2R[sub]8, -SR[sub]7, -S(O)R[sub]7, -S(O)[sub]2R[sub]7, or -S(O)[sub]2NR[sub]7R[sub]8;
each R[sub]7, R[sub]8, and R[sub]9 is, independently, H, C[sub]1-12alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-12cycloalkyl, aryl, or arylC[sub]1-12alkyl, each R[sub]7, R[sub]8, and R[sub]9 can be optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, or NO[sub]2;
when the ring system is 1-benzothiophene, R[sub]1 is C(O)OCH[sub]3, and X is -OCH[sub]2-, then R[sub]2 is not C(O)OCH[sub]3;
when the ring system is 1-benzothiophene, R[sub]1 is C(O)OH, and X is -OCH[sub]2-, then R[sub]2 is not C(O)OH;
when the ring system is thieno[2,3-b]pyridine, R[sub]1 is isopropyl ester, and X is -OCH[sub]2-, then R[sub]2 is not C[sub]1-3alkyl ester;
when the ring system is thieno[2,3-b]pyridine, R[sub]1 is C(O)OC[sub]1-4alkyl, and X is -OCH[sub]2- or -OCH(CH[sub]3)-, then R[sub]2 is not CN;
when the ring system is thieno[2,3-b]pyridine, R[sub]1 is isopropyl ester, and X is -SCH[sub]2CH[sub]2-, then R[sub]2 is not CN; and
when the ring system is thieno[2,3-b]pyridine, R[sub]1 is isopropyl ester, and X is -SCH[sub]2-, then R[sub]2 is not isopropyl ester.

35. The pharmaceutical composition of claim 25, wherein the PTPase inhibitor is chosen from at least one compound with the formula (II):

(Chemical formulae 8. See patent image)

R[sub]1 is R[sub]5, OR[sub]5, C(O)OR[sub]5, C(O)R[sub]5, or C(O)NR[sub]5R[sub]6;
R[sub]2 is R[sub]5;
X is -O-C[sub]1-3alkylene-, -NR[sub]8-C[sub]1-3alkylene-, -S-C[sub]1-3alkylene-, -SO-C[sub]1-3alkylene-, -SO[sub]2-C[sub]1-3alkylene-, -C[sub]1-4alkylene-, -C[sub]2-4alkenylene-, or -C[sub]2-4alkynylene-, wherein any of the alkylene, alkenylene or alkynylene groups can be optionally substituted with one or more halogen, oxo, imido, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, or Q;
Y is absent, -O-, or -NR[sub]6-;
R[sub]3 is H, halogen, CN, CF[sub]3, OCF[sub]3, C[sub]1-3 alkyl, C[sub]3-4cycloalkyl, C[sub]1-3alkoxy, or aryl;
R[sub]4 is A-B-E-D, where A is absent or arylene, heteroarylene, C[sub]1-6alkylene, C[sub]2-6 alkenyldiyl, or C[sub]2-6alkynyl, each A can be optionally substituted with one or more of C[sub]1-6alkyl, C[sub]2-6alkenyl, C[sub]2-6alkynyl, halogen, CN, OCF[sub]3, OH, NH[sub]2, CHO, NO[sub]2, or Q, any of the alkyl, alkenyl or alkynyl groups is optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, NO[sub]2, N[sub]3, or Q;
each A can be optionally terminated with one or more arylene, alkylene, or alkenylene;
B is absent or -NR[sub]5-, -NR[sub]7-, -N(R[sub]5)CH[sub]2-, -N(R[sub]7)CH[sub]2-, -N(R[sub]9)-, -N(R[sub]9)C(O)-, -N(R[sub]9)C(O)C(R[sub]11)(R[sub]12)-, -N(R[sub]9)C(O)C(O)-, -N(R[sub]9)C(O)N(R[sub]10)-, -N(R[sub]9)SO[sub]2-, -N(R[sub]9)SO[sub]2C(R[sub]10)(R[sub]11)-, -N(R[sub]9)(R[sub]10)C(R[sub]11)(R[sub]12)-, -N(R[sub]9)C(R[sub]11)(R[sub]12)C(R[sub]13)(R[sub]14)-, -O-, -O-C(R[sub]11)(R[sub]12)-, -O-C(R[sub]11)(R[sub]12)C(R[sub]13)(R[sub]14)-, -C(R[sub]11)(R[sub]12)-O-, -C(R[sub]11)(R[sub]12)-O-C(R[sub]13)(R[sub]14)-, -C(R[sub]11)(R[sub]12)N(R[sub]9)-, -C(R[sub]11)(R[sub]12)N(R[sub]9)C(R[sub]13)(R[sub]14)-, -C(R[sub]11)(R[sub]12)S-, -C(R[sub]11)(R[sub]12)SC(R[sub]13)(R[sub]14)-, or -C(R[sub]11)(R[sub]12)SO[sub]2C(R[sub]13)(R[sub]14)-;
E is absent or C[sub]3-12cycloalkylene, 3- to 12-membered heterocyclidiyl, arylene, C[sub]1-12alkylene, C[sub]2-12alkenylene, or C[sub]2-12alkynylene, where each E is optionally substituted with one or more C[sub]1-3alkyl, C[sub]1-3alkoxy, halogen, CN, OH, NH[sub]2, or NO[sub]2;
D is one or more H, halogen, OH, NH[sub]2, CHO, CN, NO[sub]2, CF[sub]3, or Q;
when A, B, and E are absent, R[sub]1 is C(O)OH or C(O)OCH[sub]3, R[sub]2 is H, and R[sub]3 is H or chlorine, D is not H or chlorine;
and when A, B, and E are absent, R[sub]1 is C(O)OH or C(O)OCH[sub]3, R[sub]2 is H, and R[sub]3 is H or bromine, D is not H or bromine;
each Q, independently, is -R[sub]5, -R[sub]7, -OR[sub]5, -OR[sub]7,

-NR[sub]5R[sub]6, -NR[sub]5R[sub]7, -N[sup]+R[sub]5R[sub]6R[sub]8, S(O)[sub]nR[sub]5, or -S(O)[sub]nR[sub]7, and n is 0, 1, or 2; each R[sub]5, R[sub]6, and R[sub]8, independently, is H, C[sub]1-12alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-12cycloalkyl, C[sub]1-12alkoxyC[sub]1-12alkyl, cycloalkylC[sub]1-6alkyl, 3- to 8-membered heterocycyl, heterocycylC[sub]1-6alkyl, aryl, arylC[sub]1-6 alkyl, arylC[sub]2-6 alkenyl, or arylC[sub]2-6 alkynyl, each R[sub]5, R[sub]6, and R[sub]8 can be optionally substituted with one or more R[sub]9, -OR[sub]9, -OC(O)OR[sub]9, -C(O)R[sub]9, -C(O)OR[sub]9, -C(O)NR[sub]9R[sub]10, -SR[sub]9, -S(O)R[sub]9, -S(O)[sub]2R[sub]9, -NR[sub]9R[sub]10, -N[sup]+R[sub]9R[sub]10R[sub]11, -NR[sub]9C(O)R[sub]10, -NC(O)NR[sub]9R[sub]10, -NR[sub]9S(O)[sub]2R[sub]10, oxo, halogen, CN, OCF[sub]3, CF[sub]3, OH, or NO[sub]2; R[sub]7 is -C(O)R[sub]5, -C(O)OR[sub]5, -C(O)NR[sub]5R[sub]6, -S(O)[sub]2R[sub]5, -S(O)R[sub]5, or -S(O)[sub]2NR[sub]5R[sub]6; each R[sub]9, R[sub]10, R[sub]11, R[sub]12, R[sub]13 and R[sub]14 is, independently, H, C[sub]1-12alkyl, C[sub]2-12alkenyl, C[sub]2-12alkynyl, C[sub]3-12cycloalkyl, aryl, or arylC[sub]1-12alkyl, any of the alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or arylalkyl groups is optionally substituted with one or more halogen, oxo, CN, OCF[sub]3, OH, NH[sub]2, or NO[sub]2.

2/7/21 (Item 5 from file: 654)
DIALOG(R)File 654:US Pat.Full.

5958409
Derwent Accession: 2005-048484
UTILITY
Therapeutic and prophylactic methods for neuromuscular disorders
Inventor: Whittemore, Lisa-Anne, East Walpole, MA, US
Li, Xiangping, Wayland, MA, US
Assignee: Unassigned
Correspondence Address: Finnegan, Henderson, Farabow,;Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC, 20005-3315, US

	Publication Number	Kind	Application Date	Filing Number	Date
Main Patent	US 20050014733	A1	20050120	US 2004858353	20040601
Provisional			US 60-474603	20030602	

US Classification on document (Main): 514171000
International Classification (Edition 07): A61K-315/73

Fulltext Word Count: 7446
Number of Claims: 73
Exemplary or Independent Claim Number(s): 1,23,38,53

References to Related Applications:
[0001] This application claims priority to U.S. provisional application No. 60/474,603, filed on Jun. 2, 2003, which is incorporated herein by reference in its entirety.

Abstract:
[00000] The disclosure provides methods for treating neuromuscular disorders in mammals. The disclosed methods include administering therapeutically effective amounts of a %GDF%-8 inhibitor and a corticosteroid to a subject susceptible to, or having, a neuromuscular disorder, so as to maintain desirable levels of muscle function.

Summary of the Invention:
FIELD OF THE INVENTION
[0002] The present invention relates to the field of clinical pathophysiology, and more particularly to methods for treating neuromuscular disorders, such as muscular dystrophies. The invention also relates to pharmaceutical formulations containing corticosteroids and inhibitors of growth and differentiation.

BACKGROUND OF THE INVENTION
[0003] Muscular dystrophies (MD) are progressive inherited neuromuscular disorders that are characterized by muscle wasting and weakness (Emery (2002) The Lancet, 359:687-695). Many forms of muscular dystrophies are fatal and currently incurable.

[0004] Duchenne muscular dystrophy (DMD) is the most common X-linked neuromuscular disease. The disease is caused by mutations in the DMD gene coding for dystrophin. Alteration or absence of this protein results in abnormal sarcolemmal membrane tearing. An abnormal variation in diameter of muscle fibers (atrophic and hypertrophic fibers) in proximal muscles and ongoing muscle damage are hallmarks of the disease. Damaged muscle releases the intracellular enzyme creatine kinase (CK). As a result, the serum CK levels in DMD patients are characteristically high (up to 10 times the normal). The pathophysiologic cascade is compounded by tissue inflammation, myofiber necrosis and replacement of muscle with fibrofatty tissue.

[0005] Another allelic variant of the DMD gene causes a milder form of MD known as Becker muscular dystrophy (BMD). BMD is clinically similar to DMD but the onset of symptoms occurs later in life.
[0006] Many pharmacological agents have been tried in MD but none has proved effective in arresting the course of the disease. The current modality of treatment is still in the realm of physical medicine and rehabilitation.

[0007] A number of trials using corticosteroids (e.g., prednisone and/or its derivatives) have demonstrated improvement in individuals with MD, particularly in the short-term. Although the exact mechanism by which corticosteroids alleviate the disease phenotype is unclear, corticosteroids are thought to act by reducing inflammation, suppressing the immune system, improving calcium homeostasis, upregulating expression of compensatory proteins, and increasing myoblast proliferation (Khurana et al. (2003) Nat. Rev. Drug Discovery 2:379-386). However, corticosteroids administered over time can induce muscle atrophy, which primarily affects proximal muscles-the very same muscles that are affected in DMD and BMD. The corticosteroid-induced muscle and other side effects may limit the long-term effectiveness of corticosteroid therapy.

[0008] %GDF%-8 is a member of the TGF-[small beta, Greek] superfamily and functions as a negative regulator of muscle growth. Similarly to other members of the superfamily, %GDF%-8 is synthesized as a precursor molecule, but prior to secretion, it is cleaved into the N-terminal inhibitory propeptide and C-terminal the active mature %GDF%-8. Propeptide may remain bound to %GDF%-8 thereby inhibiting the biological activity of mature %GDF%-8. Propeptide must dissociate from the complex for %GDF%-8 to bind to activin type II receptor (ActRIIB). Upon binding, ActRIIB initiates a signaling cascade, ultimately leading to the inhibition of myoblast progression. Antibody-mediated inhibition of %GDF%-8 in vivo has been shown to significantly increase skeletal muscle size in normal adult mice (Whittemore et al. (2003) BBRC, 300:965-971) and to alleviate the dystrophic phenotype in the mdx mouse model of DMD (Bogdanovich et al. (2002) Nature, 420(23):418-421).

SUMMARY OF THE INVENTION
[0009] It is one of the objects of the present invention to provide methods and compositions for treating disorders characterized by or associated with a risk of diminution of muscle function. Additional objects of the invention will be set forth in part in the following description, and in part will be understood from the description, or may be learned by practice of the invention

[0010] The present invention is based, in part, on the discovery and demonstration that, in a mouse model of DMD, treatment by administration of a neutralizing anti-%GDF%-8 antibody and prednisone is more effective in increasing muscle mass and strength relative to treatment with prednisone alone. The invention is further based, in part, on the discovery and demonstration that administration of anti-%GDF%-8 antibody with prednisone reduces prednisone induced muscle atrophy.

[0011] Accordingly, the present invention provides methods for treating neuromuscular disorders in mammals. The disclosed methods include administering to a subject susceptible to or having a neuromuscular disorder therapeutically effective amounts of at least one %GDF%-8 inhibitor and at least one corticosteroid so as to maintain desirable levels of muscle integrity or function as assessed by, for example, serum concentration of creatine kinase (CK), muscle histology, tissue imaging, activities of daily living, muscle strength and/or mass. The populations treated by the methods of the invention include, but are not limited to, patients having or at risk of developing muscular dystrophy such as, for example, DMD or BMD, and subjects undergoing corticosteroid therapy for these or other disorders.

[0012] The invention further provides methods of treating muscle weakness and methods of treating corticosteroid-induced muscle atrophy. The invention includes methods of treating cardiomyopathy.

[0013] Methods of administration and compositions used in the methods of the inventions are provided. In the disclosed methods, a %GDF%-8 inhibitor and a corticosteroid are administered concurrently or over alternating overlapping or non-overlapping intervals.

[0014] %GDF%-8 inhibitors, used in the methods of the present invention, include, but are not limited to, antibodies to %GDF%-8; antibodies to %GDF%-8 receptors; soluble %GDF%-8 receptors and fragments thereof (e.g., ActRIIB fusion polypeptides as described in U.S. patent application Ser. No.10/689,677, including soluble ActRIIB receptors in which ActRIIB is joined to the Fc portion of an immunoglobulin); %GDF%-8 propeptide and modified forms thereof (e.g., as described in WO 02/068650 or U.S. patent application Ser. No.10/071,499, including forms in which %GDF%-8 propeptide is joined to the Fc portion of an immunoglobulin and/or form in which %GDF%-8 is mutated at an aspartate (asp) residue, e.g., asp-99 in murine %GDF%-8 propeptide and asp-100 in human %GDF%-8 propeptide); a small molecule inhibitor of %GDF%-8; follistatin (e.g., as described in U.S. Pat. No. 6,004,937) or follistatin-domain-containing proteins (e.g., GASP-1 or other proteins as described in U.S. patent application Ser. Nos. 10/369,736 and 10/369,738); and modulators of metalloprotease activity that affect %GDF%-8 activation, as described in U.S. patent application Ser. No.10/662,438.

[0015] In some embodiments, the %GDF%-8 inhibitor is a monoclonal antibody or a fragment thereof that blocks %GDF%-8 binding to its receptor. Nonlimiting illustrative embodiments include a nonhuman monoclonal anti-%GDF%-8 antibody, e.g., murine monoclonal antibody JA-16 (as described in U.S. patent application Ser. No.10/253,532; ATCC Deposit No. PTA-4236); derivatives thereof, e.g., humanized antibody; and fully human monoclonal anti-%GDF%-8 antibodies (e.g., %Myo29%, Myo28, and Myo22, as described in U.S. patent application Ser. No.10/688,925; ATCC Deposit Nos. PTA-4741, PTA-4740, and PTA-4739, respectively) or derivatives thereof.

[0016] Corticosteroids, used in the method of the invention include, but are not limited to, beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone triamcinolone acetonide, and derivatives thereof.

[0017] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

Exemplary or Independent Claim(s):

1. A method of treating a mammal with a decrease of muscle function, comprising administering to the mammal a therapeutically effective amount of at least one %GDF%-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat decrease of muscle function.

23. A method of treating muscle weakness, comprising administering to a mammal a therapeutically effective amount of at least one %GDF%-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat loss of muscle strength.

38. A method of treating corticosteroid-induced muscle atrophy, comprising administering to a mammal a therapeutically effective amount of at least one %GDF%-8 inhibitor sufficient to treat the corticosteroid-induced muscle atrophy.

53. A method of treating a neuromuscular disorder, comprising administering to a mammal having or at risk of the neuromuscular disorder a therapeutically effective amount of at least one %GDF%-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat the neuromuscular disorder.

Non-exemplary or Dependent Claim(s):

2. The method of claim 1, wherein the muscle function of at least one muscle is evaluated by at least one parameter chosen from muscle mass, muscle contraction force, serum CK concentration, or muscle morphology.
3. The method of claim 1, wherein the muscle whose function is treated is chosen from at least one of gastrocnemius, tibialis anterior, quadriceps, extensor digitorum longus, cardiac muscle, or diaphragm muscle.
4. The method of claim 1, wherein treating said mammal results in increased body weight of said mammal.
5. The method of claim 1, wherein treating said mammal results in increased grip strength.
6. The method of claim 1, wherein the mammal is human.
7. The method of claim 1, wherein the corticosteroid is chosen from at least one of:
   (a) at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide;
   (b) a derivative of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide; or
   (c) a pharmaceutically acceptable salt of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide.
8. The method of claim 1, wherein the corticosteroid is prednisone or prednisolone.
9. The method of claim 1, wherein the corticosteroid is administered at a dose between 0.1 and 2.0 mg/kg/day.
10. The method of claim 1, wherein the corticosteroid is administered orally.
11. The method of claim 1, wherein the method results in treating of cardiomyopathy of said mammal.
12. The method of claim 1, wherein the administration of %GDF%-8 inhibitor and corticosteroid is concurrent.
13. The method of claim 1, wherein the administration of %GDF%-8 inhibitor and corticosteroid is consecutive.
14. The method of claim 1, wherein the %GDF%-8 inhibitor is a small molecule inhibitor.
15. The method of claim 1, wherein the %GDF%-8 inhibitor is chosen from an antibody to %GDF%-8, an antibody to a %GDF%-8 receptor, a soluble %GDF%-8 receptor, a %GDF%-8 propeptide, follistatin, or a follistatin-domain-containing protein.
16. The method of claim 15, wherein the antibody to %GDF%-8 is chosen from JA-16, %Myo29%, Myo28, or Myo22.
17. The method of claim 15, wherein the %GDF%-8 propeptide is mutated at an aspartate residue.
18. The method of claim 15, wherein the %GDF%-8 propeptide is joined to the Fc portion of an immunoglobulin.
19. The method of claim 15, wherein the %GDF%-8 receptor is ActRIIB.
20. The method of claim 15, wherein the %GDF%-8 receptor is joined to the Fc portion of an immunoglobulin.
21. The method of claim 15, wherein the %GDF%-8 inhibitor is follistatin.
22. The method of claim 15, wherein the follistatin-domain-containing protein is GASP-1.
24. The method of claim 23, wherein the mammal is human.
25. The method of claim 23, wherein the corticosteroid is chosen from at least one of:
   (a) at least one of beclomethasone dipropionate, budesonide,

cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide;
(b) a derivative of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide; or
(c) a pharmaceutically acceptable salt of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetoniden.

26. The method of claim 23, wherein the corticosteroid is prednisone or prednisolone.
27. The method of claim 23, wherein the corticosteroid is administered at a dose between 0.1 and 2.0 mg/kg/day.
28. The method of claim 23, wherein the corticosteroid is administered orally.
29. The method of claim 23, wherein the %GDF%-8 inhibitor is a small molecule inhibitor.
30. The method of claim 23, wherein the %GDF%-8 inhibitor is chosen from an antibody to %GDF%-8, an antibody to a %GDF%-8 receptor, a soluble %GDF%-8 receptor, a %GDF%-8 propeptide, follistatin, or a follistatin-domain-containing protein.
31. The method of claim 30, wherein the antibody to %GDF%-8 is chosen from JA-16, %Myo29%, Myo28, or Myo22.
32. The method of claim 30, wherein the %GDF%-8 propeptide is mutated at an aspartate residue.
33. The method of claim 30, wherein the %GDF%-8 propeptide is joined to the Fc portion of an immunoglobulin.
34. The method of claim 30, wherein the %GDF%-8 receptor is ActRIIB.
35. The method of claim 30, wherein the %GDF%-8 receptor is joined to the Fc portion of an immunoglobulin.
36. The method of claim 30, wherein the %GDF%-8 inhibitor is follistatin.
37. The method of claim 30, wherein the follistatin-domain-containing protein is GASP-1.
39. The method of claim 38, wherein the mammal is human.
40. The method of claim 38, wherein the corticosteroid is chosen from at least one of:
(a) at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide;
(b) a derivative of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide; or
(c) a pharmaceutically acceptable salt of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetoniden.
41. The method of claim 38, wherein the corticosteroid is prednisone or prednisolone.
42. The method of claim 38, wherein the corticosteroid is administered at a dose between 0.1 and 2.0 mg/kg/day.
43. The method of claim 38, wherein the corticosteroid is administered orally.
44. The method of claim 38, wherein the %GDF%-8 inhibitor is a small molecule inhibitor.
45. The method of claim 38, wherein the %GDF%-8 inhibitor is chosen from an antibody to %GDF%-8, an antibody to a %GDF%-8 receptor, a soluble %GDF%-8 receptor, a %GDF%-8 propeptide, follistatin, or a follistatin-domain-containing protein.
46. The method of claim 45, wherein the antibody to %GDF%-8 is chosen from JA-16, %Myo29%, Myo28, or Myo22.
47. The method of claim 45, wherein the %GDF%-8 propeptide is mutated at an aspartate residue.
48. The method of claim 45, wherein the %GDF%-8 propeptide is joined to the Fc portion of an immunoglobulin.
49. The method of claim 45, wherein the %GDF%-8 receptor is ActRIIB.
50. The method of claim 45, wherein the %GDF%-8 receptor is joined to the Fc portion of an immunoglobulin.
51. The method of claim 45, wherein the %GDF%-8 inhibitor is follistatin.
52. The method of claim 45, wherein the follistatin-domain-containing protein is GASP-1.
54. The method of claim 53, wherein the neuromuscular disorder is a muscular dystrophy.
55. The method of claim 54, wherein the muscular dystrophy is Duchenne muscular dystrophy.
56. The method of claim 54, wherein the muscular dystrophy is Becker muscular dystrophy.
57. The method of claim 53, wherein the mammal is human.
58. The method of claim 53, wherein the corticosteroid is chosen from at least one of:
(a) at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide;
(b) a derivative of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide; or
(c) a pharmaceutically acceptable salt of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetoniden.
59. The method of claim 53, wherein the corticosteroid is prednisone or prednisolone.
60. The method of claim 53, wherein the corticosteroid is administered at a dose between 0.1 and 2.0 mg/kg/day.
61. The method of claim 53, wherein the corticosteroid is administered orally.
62. The method of claim 53, wherein the %GDF%-8 inhibitor is a small molecule inhibitor.
63. The method of claim 53, wherein the %GDF%-8 inhibitor is chosen from an antibody to %GDF%-8, an antibody to a %GDF%-8 receptor, a soluble %GDF%-8 receptor, a %GDF%-8 propeptide, follistatin, or a follistatin-domain-containing protein.
64. The method of claim 63, wherein the antibody to %GDF%-8 is chosen from JA-16, %Myo29%, Myo28, or Myo22.
65. The method of claim 63, wherein the %GDF%-8 propeptide is mutated at an aspartate residue.
66. The method of claim 63, wherein the %GDF%-8 propeptide is joined to the Fc portion of an immunoglobulin.
67. The method of claim 63, wherein the %GDF%-8 receptor is ActRIIB.
68. The method of claim 63, wherein the %GDF%-8 receptor is joined to the Fc portion of an immunoglobulin.
69. The method of claim 63, wherein the %GDF%-8 inhibitor is follistatin.
70. The method of claim 63, wherein the follistatin-domain-containing protein is GASP-1.
71. The method of claim 63, wherein the method results in treating of cardiomyopathy of said mammal.
72. The method of claim 63, wherein the administration of %GDF%-8 inhibitor and corticosteroid is concurrent.
73. The method of claim 63, wherein the administration of %GDF%-8 inhibitor and corticosteroid is consecutive.

0005730640 **IMAGE Available
Derwent Accession: 2003-739254
Neutralizing antibodies against GDF-8 and uses therefor
Inventor: Veldman, Geertruida, INV
Davies, Monique, INV
Song, Kening, INV
Wolfman, Neil, INV
Bridges, Kristie, INV
Field, Anne, INV
Russell, Caroline, INV
Valge-Archer, Vita, INV
Correspondence Address: Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street N.W., Washington, DC, 20005-3315, US

Publication Application Filing

	Number	Kind	Date	Number	Date
Main Patent	US 20040142382	A1	20040722	US 2003688925	20031021
Provisional				US 60-419964	20021022

US Classification on document (Main): 435007100
(X-ref): 530388150
International Classification (Edition 07): G01N-033/53
Secondary: C07K-016/44

Fulltext Word Count: 18634
Number of Claims: 42
Exemplary or Independent Claim Number(s): 1,29,33,38,42
Number of Drawing Sheets: 18
Number of Figures: 18

References to Related Applications:

[0001] This application claims priority to U.S. provisional Ser. No. 60/419,964, filed Oct. 22, 2002, which is incorporated herein by reference in its entirety.

Continued Prosecution Application:

This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

Abstract:

The disclosure provides novel antibodies against growth and differentiation factor-8 (GDF-8), in particular human antibodies, and antibody fragments, including those that inhibit GDF-8 activity in vitro and/or in vivo. The disclosure also provides methods for diagnosing, preventing, or treating degenerative disorders of muscle or bone, or disorders of insulin metabolism.

Summary of the Invention:

TECHNICAL FIELD

[0002] The technical field relates to antibodies against growth and differentiation factor-8 (GDF-8), in particular human antibodies, and antibody fragments, especially those that inhibit GDF-8 activity in vitro and/or in vivo. The field further relates to diagnosing, preventing, or treating degenerative disorders of muscle or bone, or disorders of insulin metabolism.

BACKGROUND

[0003] Growth and differentiation factor-8 (GDF-8), also known as myostatin, is a secreted protein and is a member of the transforming growth factor-beta (TGF-[small beta, Greek]) superfamily of structurally related growth factors, all of which possess physiologically important growth-regulatory and morphogenetic properties (Kingsley et al. (1994) Genes Dev., 8: 133-146; Hoodless et al. (1998) Curr. Topics Microbiol. Immunol., 228: 235-272). Similarly to TGF-[small beta, Greek], human GDF-8 is synthesized as a 375 amino acid long precursor protein. The precursor GDF-8 protein forms a homodimer. During processing the amino-terminal propeptide is cleaved off at Arg-266. The cleaved propeptide, known as the "latency-associated peptide" (LAP), may remain noncovalently bound to the homodimer, thereby inactivating the complex (Miyazono et al. (1988) J. Biol. Chem., 263: 6407-6415; Wakefield et al. (1988) J. Biol. Chem. 263: 7646-7654; Brown et al. (1990) Growth Factors, 3: 35-43; and Thies et al. (2001) Growth Factors, 18: 251-259). The complex of mature GDF-8 with propeptide is commonly referred to as the "small latent complex" (Gentry et al. (1990) Biochemistry, 29: 6851-6857; Derynck et al. (1995) Nature, 316: 701-705; and Massague (1990) Ann. Rev. Cell Biol., 12: 597-641). Other proteins are also known to bind to mature GDF-8 and inhibit its biological activity. Such inhibitory proteins include follistatin and follistatin-related proteins (Gamer et al. (1999) Dev. Biol., 208: 222-232).

[0004] An alignment of deduced amino acid sequences from various species demonstrates that GDF-8 is highly conserved throughout evolution (McPherron et al. (1997) Proc. Nat. Acad. Sci. U.S.A., 94: 12457-12461). In fact, the sequences of human, mouse, rat, porcine, and chicken GDF-8 are 100% identical in the C-terminal region, while in baboon, bovine, and ovine they differ only by 3 amino acids. The zebrafish GDF-8 is the most diverged; however, it is still 88% identical to human.

[0005] The high degree of conservation suggests that GDF-8 has an essential function. GDF-8 is highly expressed in the developing and adult skeletal muscle and was found to be involved in the regulation of critical biological processes in the muscle and in osteogenesis. For example, GDF-8 knockout transgenic mice are characterized by a marked hypertrophy and hyperplasia of the skeletal muscle (McPherron et al. (1997) Nature, 387: 83-90) and altered cortical bone structure (Hamrick et al. (2000) Bone, 27 (3): 343-349). Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF-8 in cattle (Ashmore et al. (1974) Growth, 38: 501-507; Swatland et al. (1994) J. Anim. Sci., 38: 752-757; McPherron et al. (1997) Proc. Nat. Acad. Sci. U.S.A., 94: 12457-12461; and Kambadur et al. (1997) Genome Res., 7: 910-915). Studies have indicated that muscle wasting associated with HIV-infection is accompanied by an increase in GDF-8 expression (Gonzalez-Cadavid et al. (1998) Proc. Nat. Acad. Sci. U.S.A., 95: 14938-14943). GDF-8 has also been implicated in the production of muscle-specific enzymes (e.g., creatine kinase) and proliferation of myoblast cells (WO 00/43781). In addition to its growth-regulatory and morphogenetic properties, GDF-8 is thought to be also involved in a number of other physiological processes, including glucose homeostasis in the development of type 2 diabetes, impaired glucose tolerance, metabolic syndromes (e.g., syndrome X), insulin resistance induced by trauma, such as burns or nitrogen imbalance, and adipose tissue disorders (e.g., obesity) (Kim et al. (2001) BBRC, 281: 902-906).

[0006] A number of human and animal disorders are associated with functionally impaired muscle tissue, e.g., muscular dystrophy (including Duchenne's muscular dystrophy), amyotrophic lateral sclerosis (ALS), muscle atrophy, organ atrophy, frailty, congestive obstructive pulmonary disease, sarcopenia, cachexia, and muscle wasting syndromes caused by other diseases and conditions. To date, very few reliable or effective therapies have been developed to treat these disorders.

[0007] There are also a number of conditions associated with a loss of bone, which include osteoporosis and osteoarthritis, especially in the elderly and/or postmenopausal women. In addition, metabolic bone diseases and disorders include low bone mass due to chronic glucocorticoid therapy, premature gonadal failure, androgen suppression, vitamin D deficiency, secondary hyperparathyroidism, nutritional deficiencies, and anorexia nervosa. Currently available therapies for these conditions work by inhibiting bone resorption. A therapy that promotes new bone formation would be a desirable alternative to these therapies.

[0008] Thus, a need exists to develop new therapies that contribute to an overall increase of muscle mass and/or strength and/or bone density, especially, in humans.

SUMMARY

[0009] It is one of the objects of the present invention to provide safe and effective therapeutic methods for muscle and/or bone-associated disorders.

[0010] It is another object of the invention to provide methods of increasing muscle mass and/or bone strength and/or density in vertebrates.

[0011] It is yet another object of the invention to provide inhibitors of GDF-8 that are safe and effective in vivo.

[0012] Still another object of the invention is to provide human antibodies and fragments thereof that bind GDF-8 with high specificity and affinity.

[0013] Thus, methods for treating muscle and bone degenerative disorders are provided. The methods are also useful for increasing muscle mass and bone density in normal animals. Also provided are novel human anti-GDF-8 antibodies, termed Myo29, Myo28, and Myo22, and antibodies and antigen-binding fragments derived therefrom. The antibodies of the invention possess a number of useful properties. First, the antibodies are capable of binding mature GDF-8 with high affinity. Second, the disclosed antibodies inhibit GDF-8 activity in vitro and in vivo as demonstrated, for example, by inhibition of ActRIIB binding and reporter gene assays. Third, the disclosed antibodies may inhibit GDF-8 activity associated with negative regulation of skeletal muscle mass and bone density.

[0014] Certain embodiments of the invention comprise the V[sub]H and/or V[sub]L domain of the Fv fragment of Myo29, Myo28, or Myo22. Further embodiments comprise one or more complementarity determining regions (CDRs) of any of these V[sub]H and V[sub]L domains. Other embodiments comprise an H3 fragment of the V[sub]H domain of Myo29, Myo28, or Myo22.

[0015] Other aspects provide compositions containing antibodies of the invention or their antigen-binding fragments, and their use in methods of inhibiting or neutralizing GDF-8, including methods of treatment of the human or animals. The antibodies of the invention may be used to treat or prevent conditions in which an increase in muscle tissue or bone density is desirable. For example, the presently disclosed antibodies may be used in therapies to repair damaged muscle, e.g., myocardium, diaphragm, etc. Exemplary disease and disorders include muscle and neuromuscular disorders such as muscular dystrophy (including Duchenne's muscular dystrophy); amyotrophic lateral sclerosis; muscle atrophy; organ atrophy; frailty; tunnel syndrome; congestive obstructive pulmonary disease; sarcopenia, cachexia, and other muscle wasting syndromes; adipose tissue disorders (e.g., obesity); type 2 diabetes; impaired glucose tolerance; metabolic syndromes (e.g., syndrome X); insulin resistance induced by trauma such as burns or nitrogen imbalance; and bone degenerative diseases (e.g., osteoarthritis and osteoporosis).

[0016] In addition, the presently disclosed antibodies may be used as a diagnostic tool to quantitatively or qualitatively detect GDF-8 or its fragments in a biological sample. The presence or amount of GDF-8 detected can be correlated with one or more of the medical conditions listed above.

[0017] Another aspect provides an isolated nucleic acid, which comprises a sequence encoding a V[sub]H or V[sub]L domain from an Fv fragment of Myo29, Myo28, or Myo22. An isolated nucleic acid, which comprises a sequence encoding at least one CDR from any of the presently disclosed V[sub]H and V[sub]L domains, is also disclosed. Another aspect provides host cells comprising such nucleic acid.

[0018] Yet another aspect provides a method of producing new V[sub]H and V[sub]L domains and/or functional antibodies comprising all or a portion of such domains derived from the V[sub]H or V[sub]L domains of Myo29, Myo28, or Myo22.

[0019] Additional objects of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. Various objects, aspects, and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0020] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

Description of the Drawings:

BRIEF DESCRIPTION OF THE FIGURES

[0021]FIG. 1 shows that biotinylated %GDF%-8 and BMP-11 bind the ActRIIB receptor with an ED[sub]50 of 15 ng/ml and 40 ng/ml, respectively.

[0022]FIG. 2 shows inhibition of %GDF%-8 binding to the ActRIIB receptor by scFv fragments of the invention. As illustrated, the IC[sub]50 for scFv's of %Myo29%, Myo28, and Myo22 are 2.4 nM, 1.7 nM, and 60 nM, respectively.

[0023]FIGS. 3A and 3B show that preincubation of %Myo29% with biotinylated %GDF%-8 or BMP-11 at 10 ng/ml inhibits %GDF%-8 or BMP-11 binding to ActRIIB in the ActRIIB binding assay with an IC[sub]50 of 0.2-0.4 nM.

[0024]FIGS. 4B and 4C depict results of pGL3(CAGA)[sub]12 reporter gene assays, in which %Myo29% was tested. FIG. 4A demonstrates the baseline conditions, i.e., induction of the reporter gene activity by %GDF%-8, BMP-11, and activin. FIGS. 4B and 4C show that %Myo29% reduces the %GDF% -8 activity in a dose-responsive manner, with an IC[sub]50 of 15-30 ng/ml, and inhibits the biological activity of BMP-11 to the same extent. FIG. 4D illustrates that %Myo29% does not affect the activity of activin in this assay.

[0025]FIG. 5 shows results of epitope mapping for Myo22, Myo28, and %Myo29%. The epitope for %Myo29% was mapped from amino acid 72 to amino acid 88 of mature %GDF%-8; for Myo22, from amino acid 1 to amino acid 44; for Myo28, from amino acids 1 to amino acid 98.

[0026]FIG. 6 demonstrates results of a substitution analysis of the %Myo29% epitope. Residues Lys-78, Pro-81, and Asn-83 in mature %GDF%-8 appear to be important for %Myo29% binding to %GDF%-8.
[0027]FIG. 7 depicts results of an immunoprecipitation experiment performed with %Myo29% and Myo28. Conditioned medium from CHO cells expressing %GDF%-3, which were radiolabeled with [sup]35S-methionine/cysteine, was subjected to immunoprecipitation with %Myo29% or Myo28. The immunoprecipitates were then analyzed by SDS-PAGE under reducing conditions. Bands on the gel are identified as mature %GDF%-8, %GDF%-8 propeptide, and unprocessed %GDF%-8.

[0028]FIG. 8 depicts results of a pharmacokinetic study in which C57B6/SCID mice received a dose of 1 mg/kg as a single intravenous (IV) or intraperitoneal (IP) administration of %Myo29%. %Myo29% shows prolonged terminal half-life of around one week and low clearance around 1 ml/hr/kg. The fraction absorbed following IP injection is about 77%.

[0029]FIG. 9 shows comparisons of quadriceps mass in male C57B6/SCID mice treated weekly with various doses of %Myo29% (60, 10, and 1 mg/kg), or vehicle (PBS). Treatment with %Myo29%, at the 10 and 60 mg/kg dose levels for four weeks results in a statistically significant increase in muscle mass of 19% and 23%, respectively.

[0030]FIGS. 10A and 10B show gastrocnemius and quadriceps mass in female CB17 SCID mice treated weekly with various doses of %Myo29% (10, 5, 2.5, and 1 mg/kg) or PBS for four weeks. Muscle mass is increased by 10 to 20% in mice treated with %Myo29% as compared to the vehicle control.

[0031]FIGS. 11A and 11B demonstrate respectively gastrocnemius and quadriceps muscle mass in female CB17 SCID mice treated weekly with various doses of %Myo29% (10, 5, 2.5, and 1 mg/kg) or PBS for twelve weeks. Mice treated with %Myo29% show increases in muscle mass ranging from 12 to 28%.

[0032]FIG. 12 shows the front limb muscle strength, as measured by a grip strength meter, in female CB17 SCID mice treated weekly with %Myo29%

(10 and 5 mg/kg) or PBS for twelve weeks. Front limb strength is increased by 17% and 23% in mice treated with %Myo29% at 5 mg/kg and 10 mg/kg, respectively.

We claim:

Exemplary or Independent Claim(s):

1. An isolated antibody comprising an amino acid sequence substantially as set out in SEQ ID NO:n, wherein n is 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48; and wherein the antibody is capable of specifically binding GDF-8 or BMP-1.
29. A method of making an antibody that specifically reacts with GDF-8, the method comprises: (a) providing a starting repertoire of nucleic acids encoding a variable domain which either include a CDR3 to be replaced or lack a CDR3 encoding region; (b) combining the repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out in SEQ ID NO:n, where n is an integer from 31 to 48, such that the donor nucleic acid is inserted into the CDR3 region in the repertoire so as to provide a product repertoire of nucleic acids encoding a variable domain; (c) expressing the nucleic acids of the product repertoire; (d) selecting a specific antigen-binding fragment specific for GDF-8; and (e) recovering the specific antigen-binding fragment or nucleic acid encoding the binding fragment.
33. An isolated antibody against GDF-8, wherein the antibody is capable of inhibiting binding of GDF-8 to ActRIIB.
38. A method of making an antibody, comprising culturing E. coli having ATCC Deposit Designation No. PTA-4741, PTA-4740, or PTA-4739 and recovering the antibody
42. An antibody capable of specifically binding to an epitope characterized by the amino acid sequence set forth in SEQ ID NO:54.

Non-exemplary or Dependent Claim(s):

2. The antibody of claim 1, comprising the amino acid sequence of SEQ ID NO:n, wherein n is 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48.
3. The antibody of claim 1, wherein said antibody is an scFv fragment expressed by E. coli having ATCC Deposit Designation No. PTA-4741, PTA-4740, or PTA-4739.
4. The antibody of claim 1 wherein the antibody is capable of specifically binding to a protein comprising the amino acid sequence set forth in SEQ ID NO:54.
5. The antibody of claim 4 wherein at least (a) the second amino acid from the N-terminus of SEQ ID NO:54 is methionine, (b) the third amino acid from the N-terminus is serine, or (c) the fifth amino acid from the N-terminus is isoleucine, independently of each other.
6. The antibody of claim 1, wherein the antibody is human.
7. The antibody of claim 1, wherein the antibody is IgG[sub]1 or IgG[sub]4.
8. The antibody of claim 1, wherein the amino acid sequence of the antibody is modified to reduce or alter effector function.
9. The antibody of claim 8, wherein the amino acid sequence is modified at residues corresponding to amino acid 117 or amino acid 120 of SEQ ID NO:53.
10. The antibody of claim 1, wherein the antibody is IgG[sub]1[small lambda, Greek]or IgG[sub]1[small kappa, Greek].
11. A pharmaceutical composition, comprising the antibody of claim 1.
12. A method of treatment, comprising administering an effective dose of the pharmaceutical composition of claim 11.
13. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from muscle disorder, neuromuscular disorder, and bone degenerative disorder.
14. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from muscular dystrophy, Duchenne's muscular dystrophy, muscle atrophy, organ atrophy, carpal tunnel syndrome congestive obstructive pulmonary disease, sarcopenia, cachexia, muscle wasting syndrome, and amyotrophic lateral sclerosis.
15. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of Duchenne's muscular dystrophy
16. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from obesity and adipose tissue disorder.
17. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of a disorder chosen from syndrome X, impaired glucose tolerance, trauma-induced insulin resistance, and type 2 diabetes.
18. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of type 2 diabetes.
19. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need of treatment or prevention of obesity.
20. The method of claim 12, wherein the pharmaceutical composition is administered to a mammal in need for repair of damaged muscle.
21. The method of claim 21, wherein the damaged muscle is myocardial muscle.
22. The method of claim 21, wherein the damaged muscle is diaphragm.
23. The method of claim 12, wherein the antibody is administered at an effective dose chosen from 1 [small mu, Greek]g/kg to 150 mg/kg, 1 [small mu, Greek]g/kg to 100 mg/kg, 1 [small mu, Greek]g/kg to 50 mg/kg, 1 [small mu, Greek]g/kg to 20 mg/kg, 1 [small mu, Greek]g/kg to 10 mg/kg, 1 [small mu, Greek]g/kg to 1 mg/kg, 10 [small mu, Greek]g/kg to 1 mg/kg, 10 [small mu, Greek]g/kg to 100 [small mu, Greek]g/kg, 100 [small mu, Greek]g to 1 mg/kg, and 500 [small mu, Greek]g/kg to 1 mg/kg.
24. An isolated nucleic acid encoding the antibody of claim 1.
25. An expression vector, comprising the nucleic acid of claim 24.
26. A host cell, comprising the vector of claim 25.
27. The host cell of claim 26, wherein said host cell is E. coli having ATCC Deposit Designation No. PTA-4741, PTA-4740, or PTA-4739.
28. The nucleic acid of claim 24, wherein the nucleic acid comprises a nucleotide sequence of SEQ ID NO:n, wherein n is 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.
30. An antibody produced by the method of claim 29
31. A method for identifying inhibitors of GDF-8, comprising: (a) preparing a first binding mixture comprising the antibody of claim 1 and GDF-8, (b) measuring the amount of binding between the antibody and GDF-8 in the first mixture, (c) preparing a second binding mixture comprising the antibody, GDF-8, a test compound; and (d) measuring the amount of binding between the antibody and GDF-8 in the second mixture.
32. A method of increasing muscle strength or mass, the method comprising administering a therapeutically effective amount of the antibody of claim 1 to a mammal, thereby increasing muscle strength or mass
34. The antibody of claim 33 comprising the amino acid sequence substantially as set out in SEQ ID NO:n, wherein n is 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48.
35. The antibody of claim 33 comprising the amino acid sequence as set out in SEQ ID NO:n, wherein n is 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48
36. A method of increasing muscle strength, the method comprising administering a therapeutically effective amount of the antibody of claim 33 to a mammal, thereby increasing muscle strength.
37. The antibody of claim 33 wherein the antibody is capable of specifically binding BMP-11
39. The method of claim 29, further comprising fusing the nucleic acid encoding the scFv of Myo22, Myo28, or Myo22 with nucleic acids encoding the Fc portion of an immunoglobulin and expressing the fused nucleic acid in a cell
40. The method of claim 39, comprising germlining.
41. An antibody made using the method of claim 40.

? bye
07dec06 17:14:13 User219511 Session D666.6
$0.21 0.018 DialUnits File107
$17.35 1 Type(s) in Format 7
$17.35 1 Types
$17.56 Estimated cost File107
$0.81 0.024 DialUnits File128
$18.30 1 Type(s) in Format 7
$18.30 1 Types
$19.11 Estimated cost File128
$0.59 0.034 DialUnits File340
$4.22 2 Type(s) in Format 45
$4.22 2 Types
$4.81 Estimated cost File340
$4.46 0.943 DialUnits File349
$50.00 6 Type(s) in Format 7
$50.00 6 Types
$54.48 Estimated cost File349
$0.47 0.021 DialUnits File357
$7.92 2 Type(s) in Format 7
$7.92 2 Types
$8.39 Estimated cost File357
$0.36 0.029 DialUnits File399
$5.50 2 Type(s) in Format 7
$5.50 2 Types
$5.86 Estimated cost File399
$13.95 2.364 DialUnits File654
$17.40 6 Type(s) in Format 7
$17.40 6 Types
$31.35 Estimated cost File654
OneSearch, 7 files, 3.452 DialUnits FileOS
$0.53 TELNET
$142.09 Estimated cost this search
$407.50 Estimated total session cost 8.019 DialUnits
Logoff: level 05.14.00 D 17:14:13